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A BACTERIAL WILT AND ROOT ROT OF ALFALFA CAUSED BY APLANOBACTER INSIDIOSUM L. McC.[1]

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INTRODUCTION

In a previous note (*4*)[2] the senior writer recorded the finding, in northern Illinois and southern Wisconsin, of a new disease of alfalfa. This disease, chiefly vascular in character, causing as its most conspicuous symptoms a dwarfing and yellowing of the plant, a drying out of the foliage, and occasionally a wilt, has now been under observation and study for a year. The organism found in diseased plants, and with which the disease has been reproduced experimentally, has been studied by the junior writer and been found by her to be a new species of bacteria to which the name *Aplanobacter insidiosum* has been given in a brief description already published (*6*) The present paper brings together a more complete description of the disease, and such results as have been obtained in an experimental study of the relation of environmental conditions to the development of the disease, and gives a full description of the parasite.

DESCRIPTION OF THE DISEASE

Although the disease has undoubtedly passed unrecognized in the United States for a long time, it is by no means inconspicuous in character, at least in fields where it is abundant and destructive. It is not characterized by any external lesions like those occurring in the bacterial stem blight described by Sackett (*11*). The most conspicuous character of thoroughly diseased plants is a dwarf habit (pl. 1, A), there sometimes seeming to be an abnormally large number of stems suggesting a witches' broom. Dwarfing is usually more conspicuous when plants have reached one-half to two-thirds growth after the field has been cut. Dwarfed plants are usually paler in color than their healthy neighbors, and their leaves are smaller. On dwarfed stems with short internodes the leaves may be very small and curled upward and yellowed at the margins. In very vigorous fields or in plants recently infected the symptoms may be inconspicuous. Dwarfing may be slight or there may be no dwarfing.

[1] Received for publication Jan. 28, 1926; issued September, 1926. This paper is a joint contribution from two separate offices of the Bureau of Plant Industry of the United States Department of Agriculture. The fore part of the article, beginning on this page and continuing on to page 502, is by Jones, the senior writer, and is devoted especially to the nature, distribution, and control of the disease. The second part, beginning on page 502 and continuing to the end, is by McCulloch, the junior writer, and is especially concerned with the culture and morphology of the bacterium which has been assigned as the cause of the disease.

[2] Reference is made by number (italic) to "Literature cited," p. 521.

Sometimes a bleaching and drying of the foliage of a few stems or of the margins of a few upper leaves like that following frost injury is the only indication of the disease. In seed fields the disease has sometimes been found betraying itself merely by the yellowing of the margins of the oldest leaves, giving the entire plant an unhealthy appearance. In the greenhouse, infected plants have sometimes shown as first visible symptoms a mosaiclike mottling of the leaves after several hours of sunshine, with a return to uniform color at night. Extreme mottling of the foliage is followed by wilting or shriveling (pl. 2); indeed, the frosted appearance of plants in the fields seems to be the consequence of severe wilting in hot midday. The wilting of older plants has been observed by the senior writer, although it has been described as characteristic chiefly in the first crop, and rarely in the later crop. Wilting of seedlings in their first year has been seen once in the field, and this wilting seems to be the only symptom which young plants exhibit before they perish. The term "wilt," then, as applied to this disease, designates more accurately the relation of the parasite to the host—the extensive clogging of the vascular system described later—than the most conspicuous symptom of the disease in the field.

Whenever this disease has developed sufficiently to be discernible in the foliage, the taproot almost always shows unmistakable discoloration, which is readily observed when it is cut across with a sharp knife (pl. 3). The discoloration, which is yellow or pale-brown in color, is located in the outermost part of the woody cylinder just beneath the bark. The ring of discoloration is narrow at first, increasing in width with the progress of the disease. When the bark is stripped back, the woody vascular cylinder thus exposed is seen as straw-yellow to brownish-yellow in color, very different from the white or ivory-white, rather dry appearance of the same tissue in the healthy plant. This condition is found not only near the crown, but it extends to the ends of long taproots and to the larger root branches. Sometimes in plants which are growing and laying down new wood in spite of the disease, the discolored wood is found deeper than the outer wood beneath the cambium, and is seen readily only in section. In any case, the character and the location of the discoloration distinguish it from the decay of the center of the taproot found so often in older plants.

Another type of root injury which occurs sometimes in badly diseased plants is a reddish-brown, cankerlike lesion in the cortex and extending more or less deeply into the wood and seen best when the bark is stripped back from the wood (pl. 1, B). This injury is rarely observable from the outside of the root. The brown discoloration of the wood extends up and down from these lesions, which appear to mark a point of entry of the parasite. This type of injury occurs from the crown to a depth of 6 to 8 inches in the soil, and is less readily distinguished from other types of crown decay.

Dwarfing and yellowing of plants, or bleaching of the foliage, together with discoloration of the outermost woody tissue of the taproot, are the distinguishing characters by which this disease can usually be easily recognized.

The distribution of the disease in the field may be uniformly scattered, especially if the field is level and uniform, but more frequently it occurs in patches. It is always more abundant along depressions

where surface drainage takes place, and along irrigation ditches. Frequently from isolated beginnings it spreads conspicuously in the direction of surface drainage or in the direction of the flow of irrigation water.

DISTRIBUTION OF THE DISEASE

The disease is now known, locally at least, in many States where alfalfa is grown. It has been reported recently in correspondence from New Jersey, Pennsylvania, and South Carolina, by J. L. Weimer. In a preliminary survey in May and June, 1925, by Weimer and the senior writer (*5*) the disease was observed in Alabama, Mississippi, Indiana, Illinois, Wisconsin, South Dakota, Nebraska, Kansas, and Colorado. The pathogenicity of cultures of the parasite obtained from plants in all of these States, except Indiana, has been determined by inoculation of plants in the field and the production of typical disease. Recently the disease was found by M. B. Linford in Cache Valley, Utah, and by the senior writer and B. L. Richards in southern Idaho. This experience suggests that further search probably will result in discovery of the disease in greater or less abundance in many more if not all of the older alfalfa-growing districts of the United States.

PREVIOUS RECORDS AND STUDIES

References to this disease that can be identified with certainty are few and are found only in recent writing. Headden (*3*) gives an account of alfalfa failure in Colorado, which does not give a convincing description of this disease; yet representative dying plants sent to the senior writer were excellent specimens from which cultures of the parasite were obtained. Sackett (*12*) has written a description of the disease that seems unmistakable, and Durrell and Sackett (*1*) later describe it even more clearly. To be sure, the bacterial organism which these latter writers found associated with the disease appears, from the one cultural characteristic given, to be different from that described in this paper; but complete proof of the ability of their organism to reproduce the disease as found in the field was not given by them in their note (*1*), and until such proof has been obtained it is not possible to distinguish the disease found by Durrell and Sackett from that described in the present paper. Melhus et al. (*8*) record an experimental study in Iowa of water-flow interference in roots of alfalfa suffering from a wilt disease, which, from the brief description they give, appears identical with the one discussed in this paper.

ECONOMIC IMPORTANCE OF THE DISEASE

It is not possible to state with certainty to what extent this disease is responsible for decrease in alfalfa culture and for "failures" which seem to be reported in increasing numbers in several parts of the United States. In the past the dying of plants from this disease has been frequently ascribed to winter injury; and more recently, especially in Colorado (*1*), when parasitic nematodes have occurred associated with the disease, the injury has been ascribed entirely to the nematodes. Much further field study will be required before a reliable

appraisal can be made of the relative importance of winter injury and this bacterial disease wherever the two troubles occur together. In the course of a field survey made during the summer of 1925, fields of alfalfa 3 to 5 years old were found in Alabama, Wisconsin, Illinois, Kansas, Nebraska, and Colorado that were greatly reduced in stand and made unprofitable by this disease. In Kansas and Nebraska this observation has been supported by Melchers (7) and Goss (2), respectively. There is, therefore, no doubt that the disease, under certain conditions, is capable of destroying fields in localities in all of these States, yet nowhere has it been found occurring in all fields in a locality—on the contrary, sometimes in only a small percentage of them—and neither is it destructive in all of those in which it occurs. A discussion of conditions which seem to favor destructive spread of the disease is given later.

RELATION OF THE PARASITE TO THE HOST

Study of the relation of the parasite to the host—the routes by which the parasite gains entrance, the manner of its spread through the tissues, the conditions under which the disease progresses to cause the death of the plant, and the bearing which age may have on the response of plants to infection—has not reached satisfactory conclusion. Such study involves a more exact knowledge of the anatomy of the plant and the effect of environmental conditions upon its growth than is available at present. Therefore the present account of these relations is merely a statement of the more obvious features that have been observed and examined.

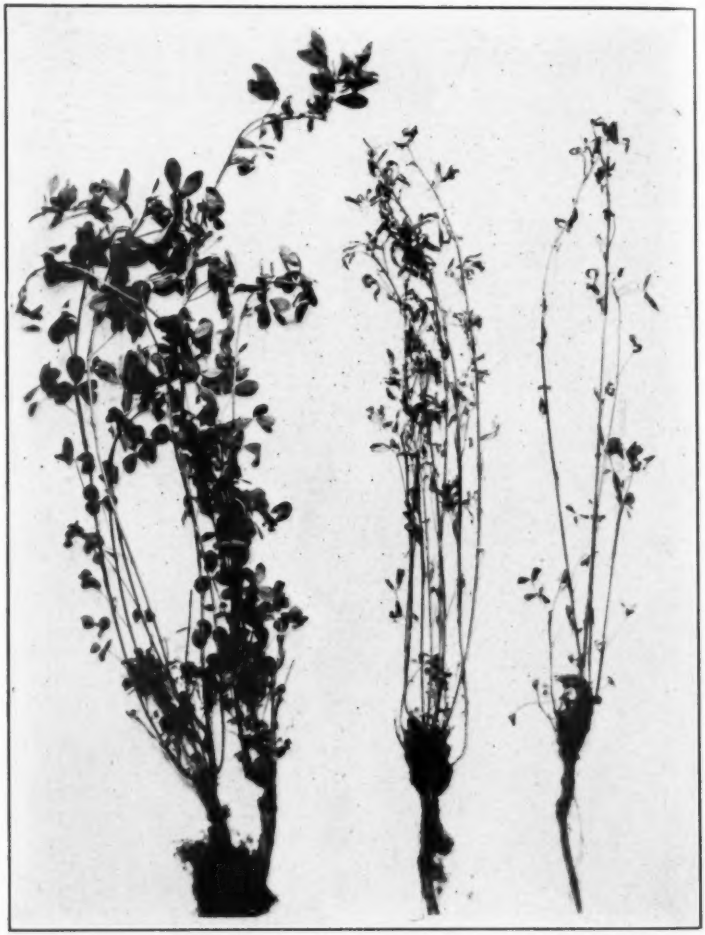
METHOD OF INFECTION

Direct inoculation of alfalfa plants with pure culture of the parasite has been made successfully by two methods, both involving wounding of the host. The first method introduces the organism directly into the vascular system of the plant; the second brings the organism into contact with wounded parenchyma through which it must pass to enter the vascular system. The organism has not yet been found to enter uninjured tissue.

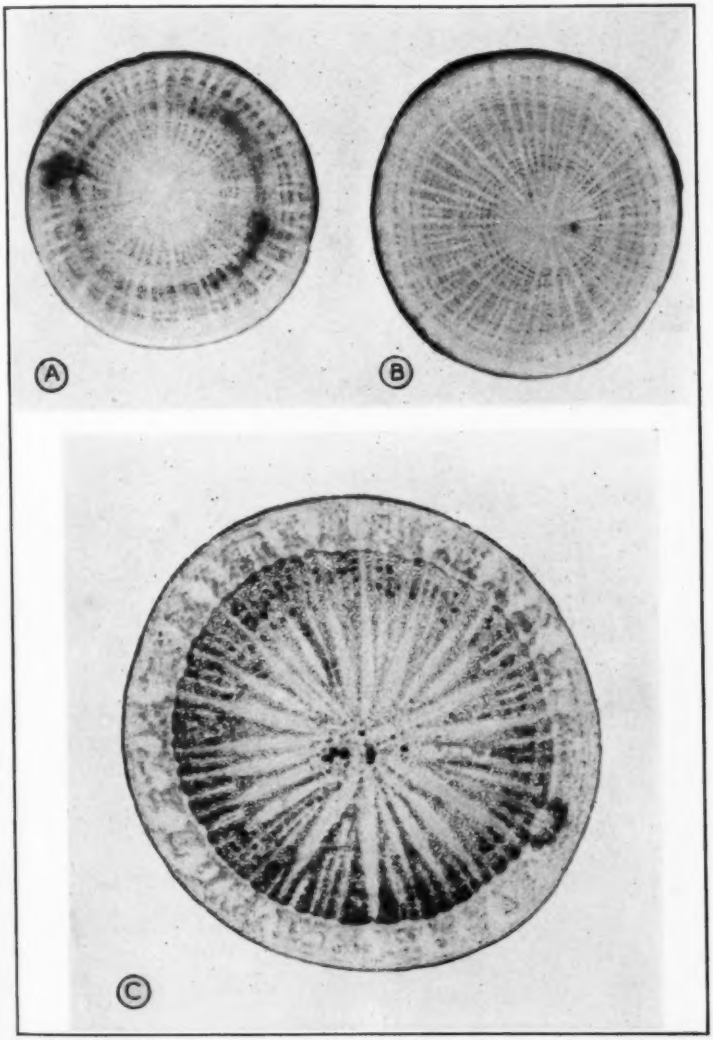
The first method, used almost exclusively in experimental inoculations thus far, takes advantage of the great avidity of the freshly cut vascular tissue of alfalfa root or stem for water. Alfalfa stems have never been observed to "bleed" when cut, but even when the soil is wet they take up water, and this water may carry bacteria in suspension a considerable distance into the plant. The conditions most favorable for absorption of water and the extreme distance to which bacteria may be carried have not been determined. Bacteria may be carried both upward and downward from cuts. In a preliminary demonstration, a vigorous shoot of alfalfa 38 centimeters tall was cut off close to the crown, the cut being made under a bacterial suspension in which the stem was left standing in the greenhouse for 5 hours before it was examined for the presence of bacteria. Razor sections from 3 centimeters above the cut showed vessels to be clogged with pale-yellow masses of bacteria, and bacteria were obtained in plates poured from the eighth centimeter of stem above the cut. In a younger stem 22 centimeters tall, bacteria were found 6 centimeters above the cut 5 hours after the stem was cut off. Similar

A. Alfalfa plant severely diseased with bacterial wilt and root rot (lower left), showing characteristic appearance in the field about two weeks after cutting. The foliage is very small and pale compared with that of the healthy plant at the right

B. Two-year-old alfalfa root infected with bacterial wilt. The bark has been cut and separated from the woody cylinder to expose abundant reddish-brown lesions, some of which appear to mark the point of entry of the parasite into the root through lesions about injured and killed rootlets



Disease symptoms obtained in the greenhouse by inoculation with a culture of *Aplanobacter insidiosum*. Two uninoculated plants are shown in the group at the left. Inoculation was made October 8, 1924. Photographed January 19, 1925. The plants were not cut back in the meantime, and therefore show none of the dwarfing that is so conspicuous at a later stage in the development of the disease

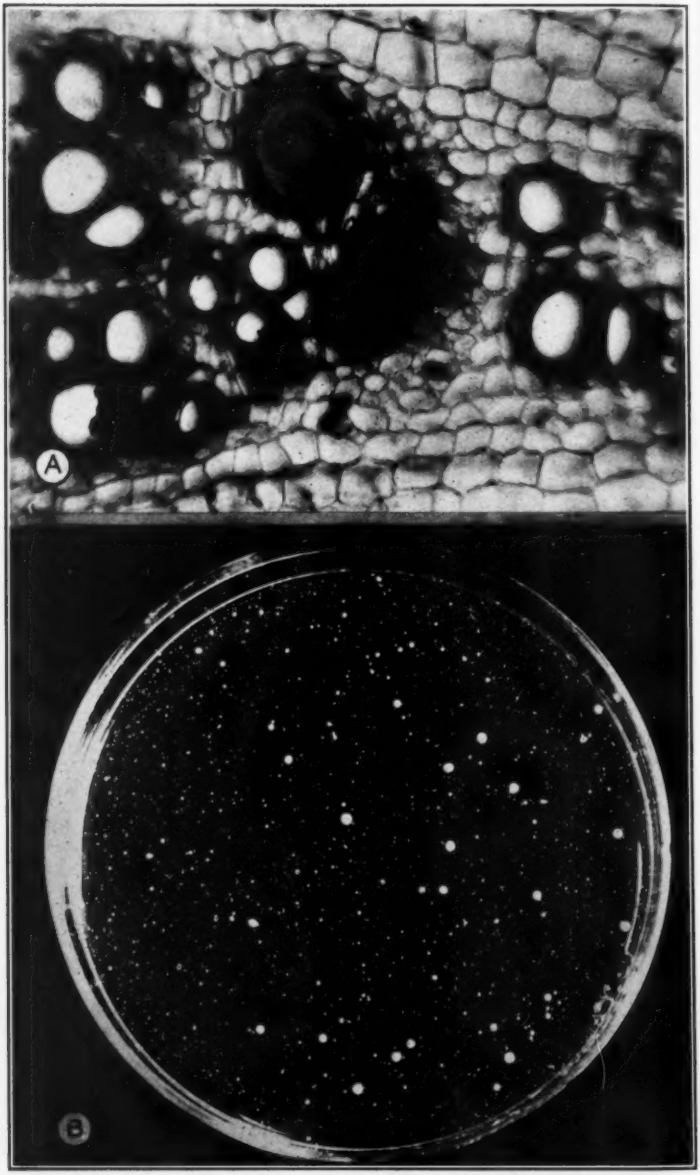


Discoloration of root in the outer wood beneath the bark characteristic of bacterial wilt and root rot

A. Cross section of an infected plant 3 years old. Note in the outer part of the wood the discoloration which in this case (as often) is not of equal intensity around the entire circumference of the root. × 3¼

B. Cross section of healthy root 3 years old. × 3¼

C. A thinner section of the diseased root shown in B photographed by transmitted light to show more clearly that the discoloration which appears diffused in B is due chiefly to the colored material in invaded vessels. × 6



A. Three vessels of the vascular system of alfalfa plugged with bacteria are shown in the center of the photograph. × 250

B. *Aplanobacter insidiosum*. Plate for isolation. Nine-day-old culture on beef agar. Note the small size of the colonies at this age. Actual size

stems cut near the top under bacterial suspension and allowed to remain in the suspension four hours, gave abundant bacterial colonies from the ninth centimeter below the cut, which was the greatest distance from the cut at which examination was made.

Bacteria are carried a considerable distance in a much shorter time than that used in the foregoing trials. Large stems of plants in late bloom growing in the field were cut off about 15 centimeters above the crown, with a razor wet with bacterial suspension, and the cut surface was quickly irrigated with a single drop of the suspension as large as the level surface would hold. This drop was immediately absorbed. Five minutes later the stump was cut off, then sterilized on the surface, and cut into pieces one-half centimeter long which were macerated and plated. An abundance of bacteria was obtained from the fourth centimeter below the top of the stump. This experience indicates that the host plant would readily take the parasite into its vascular system when wounded in mowing or cultivation. Nearly all of the inoculations made thus far have been made by cutting stems or roots under bacterial suspension or with a razor wet with bacterial suspension, and in no case has infection failed to result.

The second method of inoculation, tried with pure culture but once in the field, consisted in removing the soil from around the crown and scraping the bark of the taproot, taking care not to penetrate the wood, and then applying a bacterial suspension to the scraped surface. This inoculation was made July 17. On September 22 ten of the inoculated plants and 10 of the injured but uninoculated controls were removed for examination. The uninoculated plants had healed, and showed no deep injury. The inoculated plants, however, while showing little injury on the surface, had reddish-brown lesions in the cortex beneath the wounds extending down into the wood. Vascular infection occurred in all of these plants, sometimes only immediately above and below the discolored spot in the wood. It was thought that some of the small roots might possibly have been cut accidentally by the instrument when the taproot was scraped and that the infection might have gained entry into the taproot in that way, so slides were made of the discolored cortex to find out whether the vascular tissue of any of the small roots had been cut, but examination of the slides failed to reveal any such avenue of access for the infection into the taproot. From eight lesions isolations were made from cortical tissue only, and in seven cases abundant colonies of the parasite were obtained. From this it appears that the parasite can make its way through parenchyma from superficial wounds to the vascular tissue, causing more or less cortical crown or root rot. These reddish-brown lesions have been found in abundance in one 3-year-old field of alfalfa which was dying very rapidly from this disease, and the parasite has been isolated readily from such cortical injury. This type of injury by the parasite deserves further study.

DISTRIBUTION OF THE PARASITE WITHIN THE HOST

Whatever the manner in which the parasite gains entrance into the vascular system, it travels in that system slowly, passing through the crown from root to stem or from stem to root with equal facility.

The bacteria in the vessels may be seen in razor sections as a more or less deeply colored yellow mass filling the entire cavity (pl. 4, A). The cell wall and also the contiguous wood fibers usually remain free from color, at least for some time. The yellow material in the vessels becomes denser and darker in color with age, until the lumen of the cell is filled with a yellow gum. Whether this final material is of bacterial origin or is deposited by the plant is not known.

The vessels of the root that are filled with bacteria are usually the outermost ones, those nearest the cambium, and are more or less densely scattered around the entire circumference. In older roots they are rarely found in vessels near the center. From superficial examination it appears that the reason for this distribution is that the bacteria, whether they enter through the cortex or come down from stems, find their way into, or have the most unobstructed entrance into these outermost vessels, which anastomose freely around the entire circumference but which connect radially only rarely if at all. In the stems there appear to be no tangential connections; the bacteria in passing upward apparently follow only those vessels which they entered at the base. In fact, all of the sections of stems that have been observed have shown a diminution in the number of clogged vessels from internode to internode progressively upward.

Invasion of the stem seems to follow the maturity of the tissue. Little or no invasion has been found in young shoots, even when they show conspicuous evidence of disease, which suggests that disease symptoms are largely if not wholly the result of bacterial action in the roots. Extensive invasion of stems has been found, both in the greenhouse and the field when there was very little or no evidence of disease in the foliage. Stems grown in the greenhouse from roots inoculated by dipping ends of taproots which were cut 10 inches below the crown in bacterial suspension and grown under favorable conditions have yielded cultures of the parasite from petioles of leaves 3 feet above the point of inoculation before visible symptoms have appeared.

Does Vascular Invasion Extend to the Seed

Some of the plants described above blossomed abundantly, but even with hand pollination they set only a few seed pods. Six of these plants were examined as soon as mature, by plating the pedicel, the pod, and the seed removed aseptically from the pod. One pedicel gave a few colonies of the parasite, but none were obtained from the pods or seed. The few remaining pods were accidentally lost.

The fact that bacteria have been found in leaves and so close to seed suggests strongly that they may at times enter the seed itself, and in this way be carried to new fields in viable seed. In order to explore this possibility further some days were spent in late August and early September in seed fields in Kansas and Idaho where the disease was found in greater or less abundance in seed plants. As was expected, some of the severely diseased and stunted plants in the field produced no blossoms, while numerous others produced blossoms but set no seed. A few infected plants were found that showed but slight evidence of disease in the foliage, and were vigorous enough to mature more or less seed.

The collection of infected plants bearing seed and taken to the laboratory for study has not yet been completely examined; but thus far the parasite has not been isolated from the seed.

EFFECT OF TEMPERATURE ON THE PROGRESS OF THE PARASITE THROUGH THE PLANT AND ON THE DEVELOPMENT OF DISEASE

In a preliminary inoculation in the greenhouse, 2-year old alfalfa plants transplanted from the field early in January and now in blossom were inoculated by cutting back the stems close to the crown with a razor dipped in a bacterial suspension. Five pots containing three inoculated plants each, and two pots with uninoculated plants, were placed in each of three greenhouse compartments maintained, with only a little fluctuation, at 16°, 24°, and 28° C. Inoculation was made March 2. On March 23, no evidence of disease having appeared in the foliage, the tops were cut back. By April 2 inoculated plants in 2 pots held at 28° showed typical symptoms in dwarfed foliage, and 4 inoculated plants held at 24° showed less advanced symptoms. On May 4 all remaining plants were removed from the soil and examined. Twelve of the 15 inoculated plants that were held at the highest temperature (28°) showed more or less vascular invasion in the taproot; a like number showed no infection at the intermediate temperature (24°); while all plants held at the lowest temperature (16°) were infected, but they showed relatively little vascular invasion and no evidence of disease in the foliage at any time.

A more critical study of the effect of temperature upon the progress of the parasite through the vascular system was attempted in the Wisconsin soil-temperature tanks. Alfalfa roots similar to those used in the preceding experiment were cut off about 10 inches below the crown and inoculated by standing them in a bacterial suspension when they were transplanted from the field. Inoculation made in the root permits a more satisfactory control of temperature at the point of inoculation than inoculation at the crown. Transplanted roots placed immediately at high temperature rot easily; most of one series of plants placed at temperatures from 9° to 33° C. was lost on this account. The upper limit of temperature at which the parasite will flourish in the host has not been determined.

Another group of plants inoculated as in the preceding trial were transplanted in like manner and placed in temperature tanks held at 9°, 12°, 15°, and 18° C., to determine the lowest limit of temperature at which advance through the vessels takes place. Three plants were placed in each can, and four cans were placed at each temperature. Inoculation and planting was done January 14. At the end of January, one of the plants held at 18° had bacteria in abundance in the lower half of the root, but few in the upper half. On February 11 a number of vessels in one of the plants held at 18° were found plugged close below the crown. In one of the plants held at 12° only a few bacteria were present even 4 inches above the cut end. On March 3 bacteria were isolated from the petiole of a leaf more than 2 feet above the ground on a plant grown at 18° soil temperature. At mid-April it was no longer practicable to maintain a soil temperature at 9°. At this time one plant in this series showed slight dwarfing typical of the disease, but in none of the stems were bacteria found more than 8 inches above the crown. Thus even at this low temperature of 9° bacteria had advanced through the root into the stems, and had been able to produce some slight symptoms of disease. So there was no temperature in this series that was low enough to prevent the development of the parasite in the plant.

When all of the plants were cut back on May 10 none showed striking symptoms of disease. Shortly after this the plants were accidentally allowed to dry up. Examination showed, however, that new growth of wood outside the diseased tissue was taking place, indicating a partial or temporary recovery.

Thus it appears that bacteria pass through the vascular tissue of the root at least slowly, even at a soil temperature as low as 9° and readily at 12° or 15° C.; but under the conditions of this trial none of the plants were killed in five months—even the symptoms of disease were not striking—and new uninvaded wood growth produced on the outside of the taproot suggested that these plants were likely to live and perhaps to thrive for a long time.

Further study of the effect of temperature upon the passage of bacteria through the host tissue must be made; but it is even more important, though certainly more time consuming, to determine the effect of temperature upon the longevity of infected plants.

HOST PLANTS

The only legume growing in close association with diseased alfalfa which has shown disease with similar symptoms is sweet clover. Sackett (*12*) states that alfalfa and sweet clover are both affected in Colorado. This statement seems to be based on observation of symptoms rather than upon any experimental demonstration of the common origin of the disease in the two plants. In preliminary trials which have been made to inoculate white sweet-clover plants with the alfalfa parasite disease has not been produced.[3] A few plants of red clover and of several annual legumes have been inoculated by introducing the alfalfa parasite into the vascular system; but in no case has it made extensive growth in these plants or produced symptoms of disease.

DISCUSSION

Although a discussion of the importance of this disease and its relation to cl matic factors based on observations made during a single season as described previously is necessarily a record which may need modification later, nevertheless it may be worth while to record those impressions. Bacterial wilt of alfalfa has not been found thus far a disease of seedlings or young plants, but chiefly of plants 3 years old or older. For the most part it occurs more frequently in alfalfa fields in regions where this crop has been grown for a considerable number of years. The disease also appears to increase in abundance and also in the rapidity with which it spreads through fields and destroys plants in a direct relation to the abundance of water supplied to the surface of the soil. Thus, although the disease may occur in occasional plants in old fields where annual rainfall is as low as 20 inches, it appears rarely to be destructive until rainfall exceeds 25 inches. Nearly all of the badly diseased nonirrigated fields have been in territory with rainfall between 30 and 40 inches. When water is supplied by irrigation, the disease has been found thus far absent or very scarce where fields are subirrigated, the surface soil remaining dry during most of the summer.

[3] Since the above was written, infection of white sweet clover has been secured readily in the greenhouse, and a wilt of sweet clover produced by *Aplanobacter insidiosum* has been found in one Wisconsin field where the sweet clover seed was sown among diseased alfalf plants.

When surface irrigation is used, the disease has been found largely along irrigation ditches in the more porous soils. It is more abundant throughout fields when soil is retentive of moisture, remaining wet a relatively long time after water is applied.

If these generalizations are confirmed in following years, then it appears that this disease will never become important in the drier part of the United States where alfalfa has been most extensively grown in past years; but increasing trouble may be anticipated in the more humid regions, especially in the central Mississippi Valley, where the crop has been grown more extensively in recent years.

CONTROL MEASURES

Until the life history of the parasite has been completely traced, and until the conditions which favor infection and the rapid death of the plants are better known, control measures can be suggested only for trial. The absence or scarcity of the disease in the drier part of the alfalfa-growing area of the United States indicates that the disease is not likely to become established or injurious there in the future. Effective control is of interest in those irrigated districts where the disease has already appeared, and in the warmer and more humid States where the disease threatens to check the present expansion of alfalfa culture.

Although the transmission of the parasite within or with the seed has not yet been demonstrated, it must still be reckoned as a possibility; and therefore seed from disease-free fields should be chosen if possible. The present knowledge of the distribution of the disease is so incomplete that it can not be said with certainty that the disease is absent from any seed-growing district, although in most districts it probably occurs in only a small number of fields. Generally speaking, the climatic conditions most favorable for seed production seem unfavorable for the development of this disease, so in any case the amount of diseased seed on the market can not be large. Whether the parasite is carried with seed or not, it is undoubtedly carried in a living condition for a long time in alfalfa hay. The survival of the organism for five months in hay has been demonstrated, and beyond doubt it lives much longer. Such hay scattered in new fields could undoubtedly, under favorable conditions, be a source of infection.

After the disease has appeared in a field it seems to be distributed principally by natural agencies—e. g., the flow of surface water—agencies which can hardly be controlled. However, in one instance, the most rapid distribution seemed to be in the direction taken by the mower across a diseased strip. Presumably the mower in cutting diseased stems became smeared with the bacteria, which were carried to the cut stems of healthy plants. Spreading by the mower will undoubtedly be greatly lessened if fields are cut only when they are dry.

In irrigated fields the disease has been observed to spread with the flow of irrigation water over a group of diseased plants. When the disease has appeared in an irrigated field care should be taken to use no more water than is necessary, and any cultivation of the field which wounds the crowns when the soil is wet should be avoided.

The length of time during which the parasite will persist in the soil after a diseased alfalfa field has been plowed has not been determined.

However, disease in new plantings on old fields has been observed in several instances to have spread from old diseased crowns that survived an intervening period of cultivation. Fields where the disease has been should not be replanted to alfalfa until all the old plants which can carry such infection are dead and thoroughly disintegrated.

CULTURAL AND MORPHOLOGICAL CHARACTERS OF APLANOBACTER INSIDIOSUM L. McC.[4]

The junior writer's study of this organism began several months after the senior writer discovered the alfalfa disease in Wisconsin, and after he had made isolations and accomplished infections with it that proved it to be of bacterial origin (*4*). Bacteria isolated by the senior writer and diseased alfalfa plants were sent to Washington, D. C., where the following study of the characteristics of the organism was made in the Laboratory of Plant Pathology of the Bureau of Plant Industry of the United States Department of Agriculture.

A brief description of the organism and the name selected has already been published (*6*).[4]

The senior writer provided several lots of diseased alfalfa plants from Wisconsin. Some of these were field plants with natural infection, and others had been inoculated by him. Isolations made in the Washington laboratory from these plants consistently produced the same type of bacteria that the senior writer had isolated in Wisconsin.

In order to have definite proof of the pathogenicity of the organisms isolated by the junior writer, a number of healthy alfalfa plants growing in the greenhouse in Washington were inoculated. Typical infections were obtained in every case, and the reisolated organisms were identical in character with those used for the inoculations.

The thought that this disease might possibly be caused by *Bacterium solanacearum* or *Bacterium medicaginis* was soon dismissed, as the organism isolated from the diseased tissues showed characters distinctly unlike either of those organisms or any other described plant pathogen.

BACTERIA IN THE PLANT

In infected plants that show but slight outer manifestations of disease the vascular system often contains bacteria from the tip of the root to the very top of tall stems. Recently infected tissues show no discoloration or other unusual symptoms to the naked eye, but a microscopic examination reveals bacteria in the vessels. In older infections many vessels are filled with a yellow to brown, fine granular or gumlike substance (old bacteria?), and others are filled with the colorless bacteria. The bacteria do not diffuse very readily in the water of the mount; but in heavy infections enough bacteria escape to form a milky clouding about the sections. The bacteria are small and nonmotile; and these characters, together with the location of the bacteria in the vessels, make diagnosis of the disease comparatively easy.

[4] In Migula's (*9*) classification the genus name becomes *Bacterium*. In the classification recommended by the Committee on Bacteriological Technic of the Society of American Bacteriologists (*15*) the genus name is given as *Phytomonas*.

ISOLATION OF THE BACTERIA

In selecting tissues for isolation purposes, it is best to choose, if possible, those without dark-colored vessels. The older, dark tissues are more likely to contain secondary organisms.

Two methods have been used in making isolations. In one the woody cylinder is removed with sterile instruments without previous surface sterilization.

The other method, which is much easier and just as successful, is to take the selected root or stem (at least an inch in length), remove the surface irregularities, put it into 95 per cent alcohol for 3 to 5 seconds, then burn off the alcohol and rinse in water, then with sterile instruments remove and discard the cortex. Then, whichever method has been used, the central woody part should be cut or shredded into small bits, put into broth, and shaken well several times before plating. The agar for the isolation plates may be inoculated directly from the bouillon containing the finely divided tissues, or from dilutions. Rather heavy inoculations should be made, for although the bacteria may be present in great numbers, many of them are apparently dead, or much reduced in vitality. Usually 10 centimeters or more of uninoculated agar is poured into the plate, and then the tube of inoculated agar poured on top of this. The additional media keeps the culture from drying out while the slow-growing colonies are developing.

Plates should be kept under observation for at least 15 days. The colonies are seldom visible before the fifth day, and in a number of instances they were not visible until several days later (pl. 4, B). In one case the colonies were not visible until the thirteenth day. A temperature of 20° to 23° C. is the best temperature for growth.

Pure cultures of the alfalfa bacteria have been obtained in many isolations, but frequently there are rapidly-growing antagonistic white bacterial colonies along with them which absolutely inhibit the growth of the alfalfa bacteria within a radius of 10 to 15 millimeters. With several of these antagonistic colonies scattered over a plate, it is useless to expect any of the alfalfa colonies to appear. This intruder has been found more often in plates from roots than from stems.

The alfalfa organism has been isolated repeatedly by the junior writer from plants grown and artificially infected in Washington, and from plants sent to Washington from Wisconsin. Later in the season the senior writer sent diseased plants from Alabama, Colorado, Illinois, Kansas, and Mississippi, and the organism was isolated from all of them.

Successful isolations have been made from alfalfa stems that had been pressed and kept at room temperature for five months. There was apparently no reduction in numbers or vitality.

Colonies selected from the different isolations have been compared and studied in regard to morphology and cultural characters.

MORPHOLOGY

In the host plant and in cultural media, the organism is a short, nonmotile rod, rounded at the ends, occurring singly and in pairs. No chains have been found in any medium. Single rods are 0.7 to 1 by 0.4 to 0.5 μ; the paired rods are 1.8 to 2 by 0.4 to 0.5 μ. The

size varies considerably in any given culture, but difference of media does not seem to affect the size; nor does the age of the culture, within reasonable limits, affect the size, except that in quite old cultures there is a larger proportion of small sizes. Irregular shapes are found occasionally in most media, and in Dunham's solution about half of the bacteria are lunate or some other unusual shape.

The organism stains well with Ziehl's carbol-fuchsin, gentian-violet, Loeffler's methylene blue, methyl-violet, dahlia, and safranin. No spores have been found. Capsules are formed in most media. They are best demonstrated from young beef-agar cultures stained with Ribbert's dahlia stain.

Much time has been given to examinations for motility. Cultures of various ages grown on various media and at various temperatures have been examined. Occasional paired rods are seen which appear to have a slight motility; these swing about in a half circle, turn over, and sometimes change position slightly with reference to other rods. These motile (?) individuals are rare, and so far all attempts to demonstrate flagella have failed. With the Casares-Gil method, one colony (2001) has twice shown a few rods with a single, very short projection or line of small dots at one pole. These do not look like flagella but rather like a bit of slime. The growth is viscid, and it may be that these structures are projections or parts of the capsule. With Van Ermengem's method no better results were obtained. The organism is Gram-positive, and is not acid-fast.

CULTURAL CHARACTERS

The following cultural characters have been described from cultures grown at or as near 23° C. as possible, as this seems to be the most favorable temperature for the organism. The beef media were made from beef infusion, unless otherwise stated. The P_H values were adjusted to 6.8 or 7. Ridgway's color standards (*10*) have been used.

The organism is slow in growth; and, except on sugar media, the amount of growth is only scanty to moderate, and quite often a set of cultures fails to grow at all. This uncertain character of growth necessitated the repetition of many experiments. Solid media are more favorable for growth than liquid. In many cultures the bacteria seem reluctant to grow in the moister parts of the tube.

Beef-Agar[5] Poured Plates

On beef agar the growth is very slow, and the colonies do not attain a visible size for several days. The time of appearance depends on the vigor of the organism, the temperature, and the medium. When plated directly from diseased alfalfa tissues, in beef infusion agar of a favorable P_H value (6.8 to 7), and kept at a favorable temperature (22° to 24° C.), the colonies are usually visible with a hand lens in five days. When plates are inoculated from cultures that have become accustomed to artificial media, the colonies appear in three to four days. Under unfavorable conditions the growth is so slow that the agar often dries out before the colonies

[5] All beef media used were made with beef infusion plus 1 per cent of Difco peptone.

reach a visible size. If the medium retains sufficient moisture, colonies may appear in the plates 10 or more days after inoculation.

The surface colonies are at first white and rather thin. If well separated and with other conditions favorable, they become thicker and yellow. The surface color is pale-yellow, the reverse side being deeper in color (Naples-yellow). The colonies are circular, smooth, and shining; flat to slightly convex; margins entire. The structure is homogeneous; under the lens or microscope, finely granular. Occasionally, in unusually vigorous growth, the interior has numerous, small, irregular, transparent cracks, rather generally distributed through the colony. After a few days these fill in with the usual, fine, granular, nearly opaque growth. Submerged colonies are dense, opaque, yellow; mostly elliptical or spindle shaped; coarsely granular under the microscope. Well isolated surface colonies attain a diameter of 7 millimeters; one measuring 9 millimeters was found. In average platings the colonies are 2 to 4 millimeters in diameter when 9 to 10 days old (pl. 4, B). The growth is viscid, yet soft enough in some cases to flow over the margin of the colony when the plate is placed on edge. When old and dry, the colonies, both surface and buried, are transparent and pale-amber. Very rarely an old colony has in the center a few specks of blue color, visible only with a lens.

Beef Agar Plus 1 Per Cent Dextrose, Poured Plates

Growth is encouraged on this medium. Some surface colonies are 0.5 millimeter in diameter on the fifth day, and 5 to 6 millimeters on the ninth day. The characters of growth are the same as in plain beef agar, except that the growth is more rapid and less viscid or not at all viscid.

When two to three weeks old, if the medium has not dried out too much, some of the well-isolated surface colonies become considerably changed in color by the formation of blue and violet pigments in the growth. The brightly colored granules most often develop in the center of the colony, or in a wide border about the center. Usually there is a blue central area surrounded by a narrow violet zone. Sometimes the colony has either blue or violet alone, and sometimes both colors occur irregularly scattered through the colony. In colonies 4 to 6 millimeters in diameter the colored spot varies in size from a mere point to an area 3 millimeters in diameter, and the colors persist, even in old, entirely dry plates. The colors show best on the lower side of the colony. Seen from above through the yellow growth the colors are less vivid, often merely bluish or greenish gray. Small or crowded colonies rarely develop these colors, and even in the larger well-isolated colonies the formation of color is uncertain. In adjacent colonies that apparently have the same conditions, one may have only blue, another only violet, another both colors, and a fourth no color.

Potato Agar Plus 1 Per Cent Dextrose, Poured Plate

This is a favorable medium, and growth on it is as good or better than that in beef agar plus dextrose. Colonies often are 6 millimeters in diameter five days after becoming visible. They are almost color-

less at first, like boiled starch; the surface is smooth, and the interior is full of irregular, transparent cracks which later disappear. Blue and violet colors develop as in the colonies on beef plus dextrose.

Yeast-Agar Poured Plates

Colonies in this medium are more elevated than those on most of the other media, small crowded colonies being practically hemispherical in elevation; opaque; Naples yellow. Submerged colonies are spherical, lenticular, or angular in shape; those near the surface are usually large and often bursting out with growth on one side. This medium retains its moisture rather better than beef media, the growth continues longer, and the blue and violet colors are less likely to appear in the plate growth than in tube cultures on the same medium.

Whey-Agar Poured Plates [6]

Colonies on this medium are more elevated than those on beef or potato agars but less than those on yeast agar. The colonies enlarge rapidly and coalesce to form large areas of smooth, shining growth, which is at first nearly colorless and contains numerous transparent cracks but later becomes yellow and practically opaque. Blue and violet granules develop still later, as in beef plus dextrose plate colonies.

Beef-Agar Slants

Inoculations made from liquid cultures produce a uniform layer of growth, very thin and colorless for several days, becoming moderately thick and pale yellow. In the V there is a somewhat thicker area, and it is deeper in color (Naples-yellow). Streak inoculations made from agar cultures produce a moderately thick growth along the inoculated line, with a slow outward spread of thin transparent growth having entire, smooth margins. Growth is never abundant, and when some weeks old (at room temperature) it is thin and transparent, except in the small, thicker area in the V. Cultures at lower temperatures (4° to 7° C.) and subject to less evaporation, retain a moist, almost opaque growth for at least five months. The growth is viscid. With a loop it can be stretched out 30 to 40 millimeters before one end or the other loosens and contracts like a bit of rubber. At room temperature this viscidity gradually diminishes after about the twelfth day. There is no clouding below the V. There is no odor, and the medium does not change in color. When old, a very few cultures have had a trace of blue in the V region, and this was found only with the aid of a lens. The growth is fine granular in structure. Pseudozoogloeae have been observed in old cultures, but they are smaller and less noticeable than those in agar containing sugar.

Beef-Agar Stabs

There is a moderate amount of pale-yellow, smooth surface growth which sometimes is soft enough to flow like a thick liquid. The stab remains visible as a fine granular line of no particular character.

[6] Fresh milk. Boil gently for 5 minutes, then add drop by drop 20 per cent HCl until all the casein is precipitated. Remove the coagulum by straining through cheesecloth. Neutralize the whey and add to each liter: 300 c. c. H_2O, 3 gm. gelatin, 15 gm. cane sugar, 15 gm. peptone, 15 gm. agar. Steam ½ hour, clear with egg albumen, filter, tube, and autoclave.

Neither slant nor stab cultures in beef-extract agar gave growth as good as that on the beef infusion media, and the growth was not viscid.

Beef Agar Plus 1 Per Cent Dextrose

On this medium growth is more abundant than on plain beef agar. The thick, but not entirely opaque, smooth Naples-yellow growth covers the surface of slants or stabs, and the V of slants fills with growth. In young vigorous cultures there are small, irregular, transparent cracks in the growth. Growth extends downward between the tube wall and the medium. In stabs the needle path remains visible as a dense granular line. There is an acid reaction from the dextrose, which causes a slight greening of the medium. This acid condition disappears later, and a blue color begins to replace the yellow color of the growth until the bacterial growth in the lower part of slants is dark blue-gray.

Beef Agar Plus Lacotse

Growth is much like that on beef and dextrose, except that the blue discoloration begins earlier and becomes more abundant.

Yeast Agar

This medium is very favorable for growth. The surface becomes covered with a moderately thick, smooth, shining, Naples-yellow growth. Blue and violet flecks have been observed as early as the fourth day, but usually this discoloration does not begin until later; in some cultures no blue was seen until after five weeks. Eventually the blue granules that cause this change in color are so numerous that the growth becomes dark blue, with the original yellow color visible only at a few edges or in the lower part of the V. On old, rather dry cultures, the dark bacterial surfaces have a copper to red iridescence. In stab cultures the stab remains visible as a granular line. Numerous, spindle-shaped pseudozoogloeae develop in old cultures (pl. 5, B).

Whey Agar

Growth is more abundant on this medium than on any of the other media used. On the slants it is thick, opaque, smooth, Naples-yellow. In the V of slants, where there is a thicker accumulation, it is mustard-yellow. In young cultures the growth is full of small, irregular, transparent cracks. Heavy clouding extends along the wall below the V to the base of the tube. The cultures remain moist much longer than in other agars. Cultures kept at room temperature for three months still retained some moist areas of growth. The blue discoloration is also very striking. Large areas, and often the whole of the growth, become dark blue. The medium is slightly greened. Numerous spindle-shaped pseudozoogloeae are found in old cultures.

Potato Agar

Growth is moderate, and the blue granules develop as readily and as abundantly as they do in cultures on potato agar to which sugar has been added, but the general color is less blue. Payne's gray and slate-gray are close matches for the color of the cultures. In

some slant cultures a fugitive blue-green color was observed in the medium before the growth became visible. This color is probably of the same nature as that often seen on potato cylinders before the bacterial growth becomes visible.

Potato Agar Plus Dextrose

On this medium the growth is only moderate, being thin and colorless at first, then becoming thicker and pale yellow. The medium is greened slightly. In stab cultures the medium just below the surface (upper 4 to 5 mm.) becomes opaque, as though some growth had invaded the medium. This opaque area is irregular at the lower margin. In slant cultures there is a moderate amount of clouding below the V along the tube wall. No pseudozoogloeae have been observed. As in other media containing dextrose, blue granules develop in the growth. The blue discoloration is considerably less than in whey and yeast agar cultures, and it often consists only of rather thinly scattered specks. Other cultures may have fairly large areas of gray-blue color, and under the lens bright blue and green colors are seen.

Potato Agar Plus Lactose

There is better growth and more blue color on this medium than either the plain potato or potato plus dextrose. The whole of the growth layer becomes dark blue, with just a line of yellow along the margins or in the base of the V in slant cultures.

Alfalfa Agar

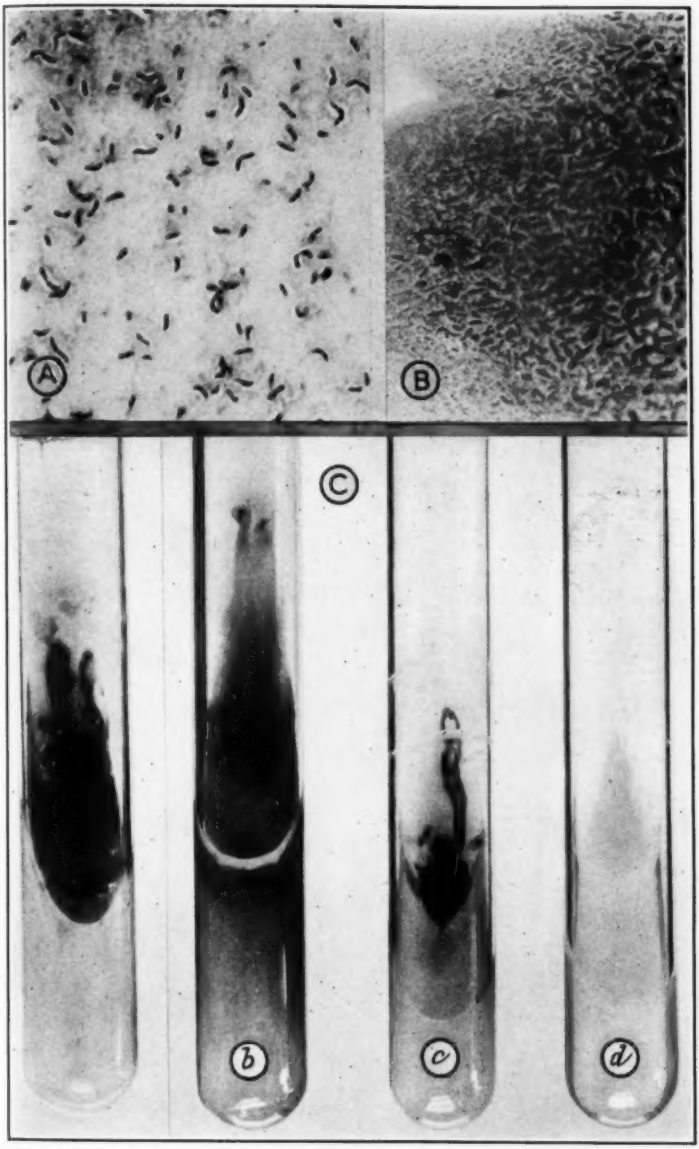
Growth is good on this medium, but not as abundant as on whey or yeast agar. Rather colorless at first, like boiled starch, becoming pale yellow. The interior is filled with small irregular cracks, producing a mottled or coarse granular effect. The surface is smooth and shining. The growth is not viscid. The medium is greened slightly more than other media containing dextrose. The blue discoloration begins early and gives a blue or green-gray color to the growth.

Lactose Agar

On a medium composed of 2 per cent lactose, 1 per cent Difco peptone, and 1 per cent agar, there is a fairly good growth, which in four to six days has considerable blue and occasionally some violet color. Agar with lactose or peptone alone failed to give more than a very scanty white growth. One reason for the scanty growth in the lactose medium may be the fact that as there is no buffer present it has not been possible to keep the medium at a P_H value favorable for the organism.

Beef Bouillon

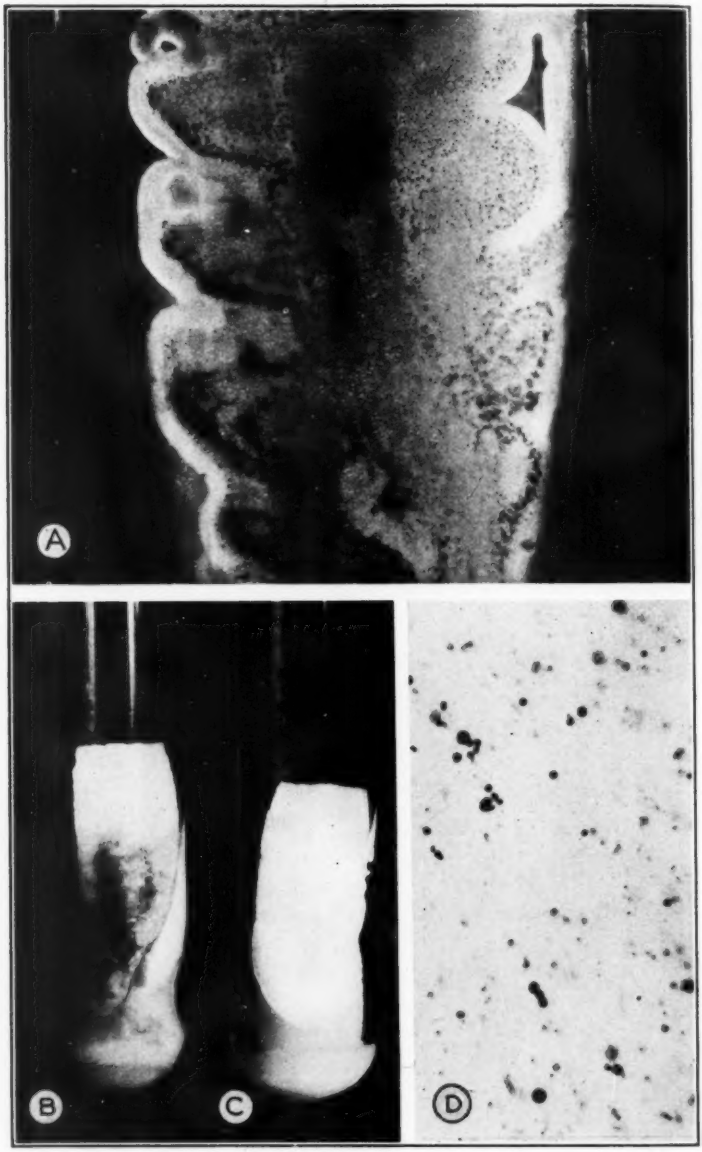
Beef bouillon of a favorable P_H value (6.8 to 7.0) rather heavily inoculated from a vigorous culture will cloud at a favorable temperature (22° to 24° C.) in 24 hours. If the medium or the temperature is even slightly unfavorable, it is not unusual for the clouding to be delayed until the third day or even later. The clouding, which never becomes heavy, is usually uniformly distributed throughout



A. *Aplanobacter insidiosum* grown on beef agar for 24 hours. Stained with Ribberts' capsule stain. × about 1,200

B. Pseudozoogloeæ in yeast-agar culture of *Aplanobacter insidiosum*. × 6

C. Blue and gray-blue color developed in cultures of *Aplanobacter insidiosum* on whey agar (a), bean agar (b), and potato-dextrose agar (c), in contrast with absence of color on beef agar (d)



A. Potato-dextrose agar slant culture of *Aplanobacter insidiosum*. Opaque blue granules have developed in the bacterial growth. × 4½

B. Potato-cylinder culture 10 days old grown at 18 to 20° C. Numerous blue granules and a good amount of growth

C. Parallel with B but grown at 23 to 25°. Growth is scanty, and there are no blue granules

D. Blue granules from a culture of *Aplanobacter insidiosum*. × 500

the broth, but if cultures under unfavorable conditions are not disturbed for some days the clouding often begins in a restricted area either against the wall or near the surface. The clouding may be considerable in these areas, while other parts of the medium remain clear until the tube is disturbed. This restricted character of growth seems to indicate a nonmotile organism. No pellicles are formed, and no rims except thin, irregular ones on cultures at low temperatures (2° to 8° C.) after eight weeks of growth. (Cultures in beef bouillon plus dextrose readily form thin, white rims.) The sediment is scanty, viscid, and slightly yellowish. After eight to ten weeks at room temperature the bouillon is usually clear. At low temperatures (2° to 8° C.) clouding persists for at least four months.

Beef-Gelatin Plates

On plates held at 20° C. tiny points of growth become visible on the fourth or fifth day. These grow slowly, requiring 12 to 25 days to reach the maximum of 3 millimeters in diameter for well-isolated colonies. The growth is at first very pale yellow and opaque; when old it is Naples yellow and transparent. Under a lens or microscope the margins are finely serrate or toothed. By the tenth day the well-isolated colonies are sunken in a shallow depression, but there is no liquid evident until about the fifteenth to eighteenth day, when the largest colonies are surrounded by depressions 7 to 8 millimeters in diameter. The depressions are shallow, entirely free from clouding and have smooth margins. A few of the larger depressions show a mere trace of liquid. After this time (10 to 15 days) both growth and liquefaction apparently cease, as there is no change, and the medium finally becomes dry. Thickly sown plates show some softening of the gelatin when about 2 weeks old. When 4 weeks old the gelatin flows only slowly when plates are put on edge.

Gelatin Stabs

If inoculated from agar cultures the stab remains visible; if inoculated from liquid cultures it is invisible. A tiny pit forms in the surface, and this gradually spreads to the walls and deepens. In two weeks the depth of liquefied medium varies from 8 to 10 millimeters. It is cloudy, and the sediment is pale-yellow; no surface pellicle or rim. After three months cultures held at 18° to 23° C. are not entirely liquefied.

Potato Cylinders

There is considerable variation in the potato-cylinder cultures, some of which is due to differences in moisture, in temperature, and in the potatoes, and some doubtless to the reaction of different colonies. In general, at room temperatures, the potato does not discolor (pl. 6, C) and growth becomes visible about the third day after inoculation, as pale yellow, shining areas. This growth spreads over a considerable part of the cylinder and becomes moderately thick, smooth, shining, and usually Naples yellow in color. If there is a large amount of water the growth is thinner, wet, and deeper yellow. However, there are exceptions in which both potato and growth discolor and follow the same course as cultures kept at lower temperatures.

At a temperature of about 16° C. growth begins as early, becomes more abundant, and continues longer than in cultures grown at room temperatures. The potato may not discolor but usually there are areas of blue, blue-green, or lavender over parts not occupied by visible growth. These colors may remain, but more often they disappear, change to dull-gray or blue, or are overgrown by the spread of the bacteria. The rather abundant yellow bacterial growth is so quickly and completely filled with tiny blue particles that the yellow color is visible only in the advancing margin of the most recent growth. This mixture of yellow and blue produces a variety of shades of green, from light, yellow-green to dark-green. During the period of most vigorous growth the predominating colors are "citrine," "buff-citrine," and "olive-green." Some areas may be distinctly lavender, violet, blue, or blue-green. Sometimes one color predominates, and sometimes several or all the colors may be found in one culture. As the cultures become older and drier the blue increases, and eventually the growth is a dark-blue, almost black. A trace of yellow remains for a time in the base of the tube, but this too disappears when all the water has evaporated. The discoloration does not invade the potato tissue.

In parallel cultures, tubes with a scanty supply of moisture produced earlier growth and discoloration than tubes containing a normal amount of water. But in the moist tubes growth continued for a longer period, and in the end the amount of discoloration equalled or surpassed that in the drier cultures.

A number of potato cylinders were made from a single large tuber. These gave comparatively uniform results in amount of growth and discoloration.

This discoloration of potato cylinders has occurred only occasionally at 23° to 25° C. and has not been seen at all in higher temperatures. Cultures grown at temperatures from 20° to 2° C. always became more or less discolored.

Alfalfa Roots

Pieces of alfalfa root were sterilized and used as a culture medium. On these the growth was scanty and of no special character. Some areas of the root, usually just above the water level, became blue-green in color and the water clouded. On the eighteenth day all the water was poured out of the tubes but no better growth resulted from this treatment. Three weeks later some of the cultures had on the wall of the tube small colonylike growths of pale yellow and many of these had bright blue centers. In a microscopic examination these blue areas were found to be composed of blue bacteria.

Milk

Five days after inoculation (at 22° to 25° C.) a pale-yellow color appears on the surface. This slowly increases to a depth of 3 to 5 millimeters and deepens in color to between "cream-buff" and "chamois." Ten to twelve days after inoculation there is a shallow layer of whey on the surface, but no noticeable curd until the eighteenth day. The whey is never very abundant, but is very clear and bright yellow (antimony-yellow is nearest color). The curd remains soft, uniform in texture, "light-buff" in color. There is no indication of

digestion of the curd in three months. The most striking character in the milk cultures is the development of color in the sediment left on the walls of the tubes by evaporation. This is at first (when cultures are 12 to 14 days old) a band of "apricot" yellow. A few days later the color is duller, and with a lens there are seen, mixed in with the yellow, numerous flecks of bright blue and lavender. These flecks of color gradually increase and some of them evidently become darker, for later the general color of the band is dark gray-blue or slate-blue, which, under the lens, shows a general mixture of bright blue, violet, gray, and greenish shades. These colors persist for at least three months. Sometimes the whole culture became pale blue-green in color on the third or fourth day after inoculation at 22° to 25° C., and earlier at 17° to 20°. This color was scarcely noticeable at the higher temperatures but was very definite at the lower temperatures. It persisted for only about 24 hours. At 17° to 20° the blue discoloration in the rims begins earlier than at 22° to 25°, and often extends over the surface of the culture, but the curdling of the milk does not seem to be hastened. Tyrosin crystals are abundant in old milk cultures.

Litmus Milk

In four to five days at 22° to 25° C. the first change occurs, this change being the appearance of yellow in the surface layers and a trace of reduction. Reduction is complete in 8 to 10 days. No reddening at any time. After five weeks the litmus color begins to return slowly. In the evaporation band bright colors develop as in the plain milk cultures.

Methylene-Blue Milk

Reduction depends, evidently, on the vigor of the inoculating material. In some tests complete reduction occurred in 24 hours, and in others it was delayed and was not complete until the eighth to tenth day. After three months the whey is pale greenish-yellow, or in tubes where the whey has evaporated there is a thin layer of greenish-blue on the surface of the cream-colored curd.

Blood Serum

Stroke cultures on Loeffler's blood serum at 22° to 24° C. produce a moderate amount of pale yellow growth, slightly rough on the surface. There is no liquefaction or clearing. The medium becomes pale cream color.

Starch Agar

There is a moderate amount of diastasic action on starch suspended in beef agar and on potato cylinders. Tests were made with iodine potassium iodide.

Congo-Red Agar

Stab culture gave a slow and scanty growth of dull reddish color in which blue granules were found in moderate numbers. The color of the medium was not affected.

Fermentation Tubes

The culture medium was a 2 per cent Difco peptone with 1 per cent, respectively, of each of the following carbon compounds: Dextrose, lactose, sucrose, and glycerin. A moderate amount of clouding developed in five days in the open ends. The amount of growth was equally good in all of the cultures. No gas was formed. Clouding remained definitely limited to the open end. Titrations made on the fifth, tenth, and sixteenth days showed acid production increasing in all for the first 10 days. After the tenth day there was a continued increase in acid in the dextrose, sucrose, and glycerin, but in the lactose a return to the original P_H value.

These cultures gave negative results when tested for ammonia.

Sterile milk in fermentation tubes did not produce gas. The milk in the open ends reacted as other milk cultures. In the closed end the milk remained unchanged for at least two months.

Nitrate bouillon in fermentation tubes clouded moderately well in the open ends. The closed ends remained clear. No gas was produced. Tests for ammonia and for nitrites were negative.

Fermentation of Carbohydrates

Brom-cresol purple was used instead of litmus, as the indicator in beef agar plus 1 per cent, respectively, of the following: Dextrose, lactose, sucrose, galactose, and glycerin. The lactose agar showed only slight indications of acid formation, and this soon disappeared. In all the others the acid formation was evident in three to four days and was stronger than in lactose. In the dextrose, sucrose, and galactose the greenish-yellow color indicating acid disappeared in two to three weeks, but in the glycerin this color had not disappeared in three months. Growth was abundant on all these cultures. One of the six colonies (2021 Wis.) used in the tests always gave a stronger and longer continued acid development than the others.

Reduction of Nitrates

Cultures in nitrate bouillon and in nitrate bouillon plus sugar gave good growth. Tests for nitrite were made according to the method recommended in the Manual of Methods (*13*). No trace of reduction occurred in any of the several experiments. Tests for ammonia were negative.

Indol

Tests were made on cultures in Dunham's solution, peptonized Uschinsky's solution, and in a medium containing peptone, disodium phosphate, and magnesium sulphate. None of these produced any indol. *Bacillus coli* grown in the same media gave good indol reactions.

Aerobism

The alfalfa bacteria do not grow if deprived of oxygen. Yeast, whey, and beef agars known to be favorable for growth were inoculated, some as "shake" cultures and others heavily inoculated on the surface, which was then covered by thick layers of agar. No growth occurred except on the surface.

Stabs remain visible if the inoculation is heavy and from agar cultures, and in some cases there may be slight growth in the upper part of these stabs. Stab inoculations from liquid cultures are not visible.

No clouding occurs in the closed arms of tubes containing dextrose, sucrose, lactose, or glycerin.

Toleration of Sodium Chloride

A medium of peptone-beef bouillon of P_H value 6.8, and containing, respectively, 2, 3, 4, and 5 per cent of chemically pure sodium chloride was inoculated from 6-day-old beef-bouillon cultures. After four days the 2 and the 3 per cent salt bouillons showed clouding, which increased until the cultures were clouded as well as the plain bouillon controls. Examinations were made for chains but none were found. No growth occurred in the 4 or the 5 per cent salt bouillons.

Toleration of Acid and Alkali

This organism grows best in media having a P_H value of 6.8 to 7. In peptonized beef bouillon with sodium hydroxide as the alkali and hydrochloric as the acid, the optimum is about 6.8 (+14 Fuller's scale). Its limits of toleration are P_H 5.6 (+26 Fuller's scale) on the acid side, and 8.2–8.4 (0 to −2 Fuller's scale) on the alkali side.

Ammonia

Cultures grown in beef bouillon, nitrate bouillon, yeast agar, milk, whey agar, and various other media of various ages, gave negative results in ammonia tests made with Nessler's solution.

Hydrogen Sulphide

This organism does not produce hydrogen sulphide. Lead-acetate agar made with beef infusion and with a P_H value of 6.8 was used in the tests, as both the beef extract and the P_H 7.6 recommended in the Manual of Methods (*13*) are unfavorable for this organism. Other tests were made by suspending lead-acetate paper over cultures in various media.

Uschinsky's Solution

In three out of four experiments this medium showed a very faint milky color in six to eight days after inoculation. This clouding (?) did not increase. Probably a slight growth occurs in this medium if all the conditions for growth are favorable.

Fermi's Solution

In four experiments there was no sign of growth, except a slight clouding from one colony (2021 Wis.) in one experiment.

Cohn's Solution

No trace of growth.

Pigment Formation

The color of the alfalfa bacteria on solid media is pale yellow (Naples yellow). In quite young cultures or in thin layers it is white or almost colorless. When grown at lower temperatures (2° to 20° C.) the color is somewhat deeper and has a tinge of orange (apricot to antimony-yellow). This deepening of color is particularly noticeable on potato cylinders and on agar containing sugar.

On most media there appear *in* this yellow growth, after a variable length of time, areas of darker colors, sometimes as small isolated streaks or spots, sometimes as fine specks generally distributed, which may remain restricted and make little or no increase. More often the darker color is rather evenly distributed in a considerable part of the growth, changing it to a bluish or greenish gray, often quite dark in tone, the normal yellow color remaining only in the margins of most recent growth (pl. 6, A). Examination with a hand lens shows the presence of numerous small dark particles in the growth, and also bright blue and violet colors that are not always plainly visible to the naked eye. The colors and their intensity vary somewhat in different isolations, in different media, and at different temperatures. At room temperatures the amount of discoloration is only moderate, except in some media very favorable for its development. At higher temperatures it has been observed only very rarely. At temperatures from 2° to 20° C. (from 15° to 17° seems most favorable) the discoloration increases until it entirely, or in large areas, replaces the original yellow color of the growth.

On potato cylinders there is considerable diversity in the color produced. Some of the variation is due to the difference in potato tubers. When a number of cylinders were made from one large tuber the results were more uniform. In general, there is first, before the growth is well established, a green or blue discoloration that seems to be due to a change in the potato rather than to bacterial growth. This color usually fades or sometimes is later covered by bacterial growth. The rather thick layer of growth is very quickly changed to various shades of yellow-green, yellow-brown, blue, or violet, or a mixture of all these colors, with the normal yellow color only at the extreme margins. Even these margins are eventually invaded, and the final result is a dark blue, almost black, growth. The interior of the potato is not discolored.

On potato agar and on various nutrient agars with sugar added, and on agar containing only 1 per cent peptone and 2 per cent lactose, the same colors develop as on potato cylinders, but there is less olive-green and more bright blue and violet. On agar that contain only peptone or only lactose, the growth is very scanty and no blue color develops. On agar containing 1 per cent peptone and $\frac{1}{10}$ per cent lactic acid (P_H 7.0), the scanty, white growth had no trace of blue. In plate colonies and in streak cultures in tubes the blue usually occupies the central part with a surrounding band or line of violet. The extreme margin of growth on agar is not changed until after growth ceases, and sometimes it never has the blue color. The colors remain very bright as long as the cultures are moist. The blues vary from a strong bright blue to dark blue, and the violet ranges from pale lavender to dark purple. In tube cultures the whole surface of growth often is uniformly dark blue in color.

The surface is usually smooth, and often has a brilliant iridescence of copper, bronze, or red-gold color. As the media dries the colors become duller, but even in cultures several months old there are isolated areas of very bright color. The violet often disappears, sometimes changing to green.

Plate cultures with a restricted supply of food and moisture usually show the discoloration somewhat earlier than tube cultures.

Cultures on the usual nutrient beef agar only rarely develop a trace of blue or blue-gray. A few tube cultures and a few plate colonies have had tiny blue spots, too small to be seen without a lens.

Milk cultures have on the walls of the tubes a wide band composed of particles left there by evaporation. This material seems too far away from the surface of the culture to be affected, but evidently there is some bacterial action in it, for this band (which in check tubes remains dull white) becomes dark gray-blue. A hand lens shows that this colored band is composed of numerous small mosaic-like bits of bright blue, violet, and gray. The milk which eventually curds does not develop any of these colors. Litmus milk cultures have a similar band on the tube wall, but its change in color is less striking as it is always more or less obscured by the litmus.

Cultures in beef bouillon plus dextrose, lactose, sucrose, or galactose, form white rims on the tube wall. In the rim of the lactose medium, blue and violet colors develop, but only in slight amount. No color has been found in the bouillon cultures containing dextrose, sucrose, or galactose.

A microscopic examination of the discolored growth in all these cultures shows that the color is due to the presence of great numbers of small, opaque, blue bodies, mostly spherical but some oval. These vary in size from mere points to bodies 9 μ in diameter (pl. 6, D). The greater number are less than 3 μ. There are masses 10 to 25 μ in diameter, but these are irregular in shape and seem to be composed of small spheres. The spheres seen singly and widely separated are very dark and opaque and not very definitely blue. When a number are massed together they have a beautiful bright blue color.

In addition to the opaque blue spheres there are smaller translucent spheres and rods of blue and violet. These latter are similar to the bacteria in size, shape, and appearance, and there seems no reason to doubt that they are bacteria. There are also great numbers of normal colorless bacteria in the mounts.

These spheres begin to develop first in isolated groups, gradually spreading out until they are rather evenly and thickly distributed in the cultures. They develop only on the surfaces exposed to the air.

Of the 40 or more colonies that have been under observation in subcultures, all have developed this pigment but not always to the same degree. Yeast, whey, alfalfa, and beef agars containing sugar, potato agar both with and without sugar, potato cylinders, and milk, are media very favorable for this color change. Of the sugars used in the media, lactose seems most favorable, with dextrose, galactose, and saccharose following in the order named. Agar containing only peptone and lactose produces the color. Sometimes the discoloration becomes visible in so short a time (two to six days) that it can

scarcely be caused by old age. Usually the change does not occur until the growth has reached and passed its maximum, and it is more likely to begin in the oldest center of growth. Transfers made from various parts of the same culture show that bacteria taken from the discolored areas are very much slower in growth than bacteria taken from the normal yellow areas. Often they do not produce any growth.

Exposure to direct sunlight has no noticeable effect on the pigment. Cultures, also thin layers of the blue granules spread on glass, were kept in a south window for a number of days, but no change in color occurred, and a microscopic examination showed the blue granules and smaller violet bodies to be unchanged in appearance.

When the blue growth was mixed with water the water became pale blue. In a corked vial this color persisted for six weeks. The addition of an equal amount of 4 per cent NaOH did not affect the color. A small amount of H_2SO_4 caused the color to fade entirely. After being in water six weeks the blue granules seemed unchanged.

Cold water does not dissolve the granules.

Heated over an open flame in water, no effect.

Heated to dryness over flame, no effect.

Boiled in water in test tube for five minutes, no effect.

In 95 per cent alcohol; not dissolved and alcohol not blued.

In 50 per cent alcohol plus a small amount of NaOH, heated, all the granules disappeared.

In 50 per cent H_2SO_4 the granules dissolve, leaving a violet stain, which later disappears.

In 3 per cent HCl; no change.

In glacial acetic acid; no change.

In 10 per cent NaOH the granules slowly dissolve; 5 to 10 minutes are required for the disappearance of average-sized granules; the action is quiet and no trace of color remains.

Mellon's reagent has no visible effect on the granules.

Temperature Relations

The maximum temperature for growth is 31° C. for some isolations, and 28° to 30° for others. The minimum is somewhere below freezing, and the optimum between 21° and 24°. There is considerable variation in different isolations and in different media. Of the different isolations tested only two grow at 31° (colonies 2021 and 2027 isolated by the senior writer at Madison, Wis.), and these usually only on solid media at this temperature, the growth becoming visible about the sixth day. Occasionally a beef-bouillon or Dunham's-solution culture of these two colonies clouds at 31° C.

The other isolations grow at 28° to 30° C. on solid media, forming tiny, isolated colonies on the slants in 8 to 10 days. Beef-bouillon cultures seldom clouded, even at 27°. In these experiments the beef bouillon and the beef agar were equal in P_H values, in amount and kind of inoculation, and were in the same incubator at the same time. Dunham's solution clouds more readily than beef bouillon, and some of this group produced growth in it at 29°; while parallel cultures in beef bouillon produced no growth. Parallel cultures of these were made on beef agar and in beef bouillon and kept at 31° for 15 days. Not a trace of growth occurred. The cultures were then left at

room temperature where all the agar cultures later produced normal growth, but only one bouillon culture clouded.

In parallel cultures of beef bouillon and of Dunham's solution, adjusted to the same P_H, the Dunham clouds somewhat earlier than the beef bouillon, at room and higher temperatures. At lower temperatures there is less difference in the time of clouding. The optimum and the maximum temperatures are higher in Dunham's solution than in beef broth of the same P_H value.

The organism very plainly does not like temperatures above 27° C., but if growth does start it is able to continue and eventually becomes fair in amount. Some cultures on whey and yeast agar at 27° to 28° have had, after 15 days, growth equal in amount to cultures grown at 22° to 25°.

These maximum temperatures for growth have been with media having a P_H value known to be favorable for growth, and with rather heavy inoculations from well developed, vigorous cultures. If the media is even slightly unfavorable, or the inoculum too old, too young, or too scanty in amount, no growth occurs at the maximum temperatures as stated. Beef bouillons and agars with 1 per cent dextrose and whey and yeast agars are more likely to give growth at the higher temperatures than plain beef media.

Cultures of the Alabama and Mississippi strains were kept at 26° to 31° C. for 45 days. No growth occurred. Another lot at 31° to 34° gave no growth in 80 days. Controls at 23° showed growth in two days.

The minimum temperature for growth is below 1° C. Both solid and liquid media known to be favorable for growth were kept over night at 6° to 7° C. The next morning they were inoculated quickly and in less than an hour placed in a temperature of −1.66° C. Control cultures at 28° to 25° developed good growth in five days. After 33 days the cultures at −1.66° were examined and a fair amount of growth found on the solid media (whey agar and alfalfa agar). Beef bouillon plus 1 per cent dextrose cultures had no visible growth, but all clouded within nine days after removal to 23° to 25° C.

At 1.5° to 2° C. beef bouillon and Dunham's solution cultures cloud in six to seven days. The growth continues and eventually is as good as at the optimum temperature. Cultures on beef agar and potato cylinders also grow well at 1.5° to 2°. The beef-agar cultures had after three months heavier growth than cultures ever produce at room temperatures. The retention of moisture doubtless contributes to continued growth.

Up to the present it has not been possible to determine exactly the optimum temperature for growth, as this optimum lies between the ranges of the available cold chambers and incubators. Room temperatures do not remain constant long enough for good comparisons with this slow-growing organism. Parallel sets of cultures were grown in four different locations with temperatures of 16° to 17°, 19° to 20°, 22° to 23°, and 23° to 26° C. Often when growth was unusually slow in starting all of these would show clouding at the same time, and occasionally clouding showed first at 19° to 20°, or even at 16° to 17°. When the growth was more normal, clouding was visible first in the cultures at 22° to 23°, and these continued to have best growth for several days, although the others usually clouded very soon after. From about the fourth to the tenth day after growth

begins, the cultures at 19° to 20° and 16° to 17° are more heavily clouded than those at higher temperatures. After this, however, all are equal in amount of clouding. The 23° to 26° temperatures seemed always less favorable than the 22° to 23°.

Cultures on solid media have given about the same results as the broth cultures. Growth continues longer and eventually becomes more abundant at the lower temperatures, probably because the media does not become dry so readily.

VITALITY

FREEZING

The freezing of freshly-inoculated beef bouillon cultures for two hours caused a slight reduction in the number of viable bacteria. Sixteen hours' freezing killed all the bacteria in one isolation (2001), and 89 per cent in another (15). In another experiment two hours freezing caused a slight reduction, and 24 hours an 89 to 93 per cent reduction.

Beef-bouillon cultures 3 days old were frozen for 16 hours without causing any noticeable reduction in the number of viable bacteria, but they were slower in growth than before freezing.

SUNLIGHT

This organism is not easily killed by exposure to direct sunlight. Four experiments indicate that one hour's exposure kills only a small proportion. But the colonies were slow in appearing. Exposures of one and one-half, two, and three hours either entirely killed or greatly reduced the vitality of all the organisms, on the exposed side of the plate. Occasional colonies appeared even after two hours, but none after three hours exposure. In all the sunlight tests there was observed a slow advance of growth in the form of tiny colonies, from the protected side of the plate into the medium on the exposed side. This sort of growth advanced sometimes as much as 10 millimeters. All the sunlight tests were made with the cover on the poured plates. The plates were placed so that the sun's rays were perpendicular to them.

IN CULTURE MEDIA

This organism has been under the junior writer's observation only seven months, too short a time to learn its limits of vitality on artificial media. Five-month-old cultures grown and kept at room temperature are still alive, but are slow in renewing growth when transferred. Cultures grown and held at lower temperatures, 2° to 10° C., for five months seem as vigorous as young cultures. There seems to be no difference in the vigor of cultures on liquid and solid media.

DESICCATION

To date the extreme limit of vitality after drying on cover glasses is 23 days. The greater number have shown no growth after drying for five days.

LOW TEMPERATURE

At 1.5° to 2° C. growth is visible in liquid media in 7 days, and on solid media in 15 days. In one month or less, visible growth develops at −1.6° C. (29° F.).

HIGH TEMPERATURES

Beef-bouillon cultures kept for 10 minutes in a water bath at 52° C. were killed. Beef-bouillon cultures kept at 35° to 36° for 10 days were killed.

Some beef-agar cultures held at temperatures of 28° to 32° C. for 23 days produced a few very tiny colonies on the slants. When removed to a temperature of 22° to 25° the growth increased, in spite of the rather dry surface.

Some potato-cylinder cultures at 28° C. for 47 days produced a scanty amount of growth. When removed to a temperature of 22° to 25° growth became nearly normal.

The following cultures made no growth at the temperatures given, but were not killed as was evident from the growth that developed after the cultures were removed to 22° to 25° C.:

Beef bouillon, 28° to 32° for 23 days.
Beef agar + 1 per cent dextrose, 30° to 32° for 14 days.
Potato agar + 1 per cent dextrose, 30° to 32° for 14 days.
Potato cylinders, 30° to 32° for 14 days.
Potato cylinders, at 28° for 24 days.
Potato cylinders, 33° to 37° for 17 days.
Whey agar, 29° to 30° for 11 days.
Whey agar, 31° to 33° for 11 days.

Some beef-bouillon cultures held at 35° to 36° C. for three days were then removed to 24°. Growth appeared first in these cultures 9 to 16 days after removal to the lower temperature.

Some beef-bouillon cultures held at 35° to 37° C. for five days and then removed to 24° never produced growth.

Whey-agar slant cultures kept at 30° to 32° C. for seven weeks did not grow. These were then removed to a temperature of 22° to 23° C. but no growth had developed eight weeks later.

Some beef-bouillon cultures were also at 30° to 32° C. for seven weeks, then at 22° to 23° for eight weeks. No growth developed.

TECHNICAL DESCRIPTION

APLANOBACTER INSIDIOSUM L. McC. (*6*)[7]

A nonmotile rod with rounded ends; single or in pairs, no chains have been found; single rods 0.7 to 1.0 × 0.4 to 0.5μ; capsules present; no spores; gram-positive; not acid fast. On peptone-beef agar the colonies are round, smooth, shining, flat to slightly raised, amorphous, white becoming pale yellow; in beef broth, moderate, uniform clouding without rim or pellicle; trace of growth occurs in Uschinsky's solution; no growth in Fermi's or Cohn's solution; gelatin slowly liquefied; blood serum not liquefied or cleared; optimum temperature about 23° C., maximum 28° to 31°, minimum below 1°, thermal death point 51° to 52°; no indol, ammonia, or hydrogen sulphide produced; aerobic; moderate diastasic action; soft curd forms in milk in 18 to 20 days; casein is not digested; blue and violet pigments develop in the evaporation band on wall of milk cultures; growth yellow on most culture media, but on potato cylinders and on agar containing sugar, opaque dark-

[7] See footnote 4, p. 502.

blue granules develop in the growth in sufficient numbers to change the color to various shades of blue and green; litmus reduction complete in 8 to 10 days; nitrates are not reduced; acid without gas produced from sucrose, dextrose, lactose, galactose, and glycerin; pathogenic on *Medicago sativa*, filling the vascular system and causing death of the plants.

According to the descriptive chart of 1920 indorsed by the Society of American Bacteriologists (*13*), its group number is 5331–31135–1222.

SUMMARY

The most serious and threatening disease of alfalfa in some irrigated districts and the more humid areas of the United States appears, from a study conducted largely in 1925, to be the bacterial wilt and root rot. The causal organism (*Aplanobacter insidiosum* L. McC.) is described fully (for the first time) in this paper.

The disease is chiefly vascular in character, the bacteria passing from the taproot to each succeeding weakened crop of stems until the plant is killed. The most conspicuous symptom is a dwarfing of severely diseased plants and pale color of foliage, and small, narrow leaflets yellowed and curled at the margins. The taproot when cut shows yellow or brown discoloration beneath the bark.

The disease is now known in many localities from New Jersey to Idaho and south to Mississippi.

Artificial inoculation of alfalfa plants has been accomplished only through wounds, introducing the parasite directly into the vascular system through cut stems, or into cortical tissue of roots whence it makes its way into vessels. In either case it enters the outermost vessels of the taproot, which are soon filled with a pale yellow mass, becoming deeper in color and more dense and gummy in consistency with age. This vascular invasion may extend to the veins of the leaves, but has not yet been demonstrated in seed.

The bacteria make some progress in filling vessels in the taproot at a temperature as low as 9° C. Symptoms of disease and the death of infected plants appear to be hastened by retardation of root growth brought about by high soil temperature or frequent cutting of tops.

This parasite has been found producing a wilt disease in *Melilotus alba*.

Control measures are suggested.

The bacteria have been isolated from fresh roots and stems, also from stems kept in a dry atmosphere for five months.

In culture media the organism grows rather slowly. Isolation plates seldom show growth in less than five days. Potato or some sugar in the medium encourages growth.

In culture media the bacteria grow best at about 23° C., but they also grow well at lower temperatures. Growth is able to begin and to continue at 1°. Growth seldom begins at 31°, but once started it is able to make some progress.

On solid media the growth is yellow, but on most media this yellow color is soon obscured by the formation in the growth of numerous small blue particles which change the color to various blues and greens, blues predominating.

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BUD SPORTS IN SWEET POTATOES[1]

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In the fall of 1923, in a planting of the Haiti variety of sweet potato (*Ipomoea batatas Poir*) in Delaware, one plant was found which produced three red and three yellow roots, as shown in Plate 1. The Haiti is normally a red variety, marked with small oval or elongated whitish to yellow spots which vary somewhat in size. (pl. 1).

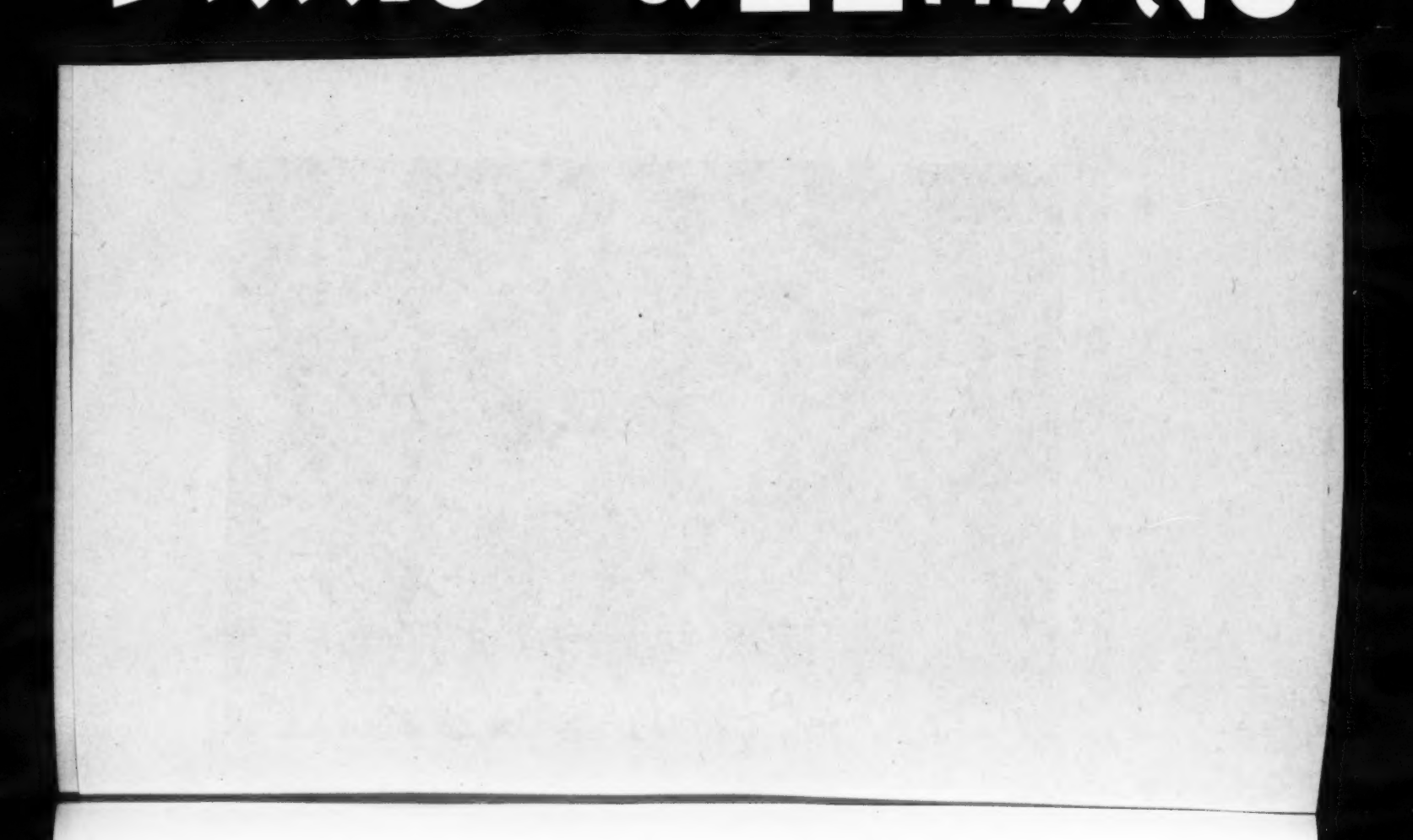
No information seems available as to the origin of the Haiti variety—that is, whether it originated as a bud sport, a mutation, or as a result of hybridization. In the section of the United States in which this plant was found, flowers are rarely produced and seeds never, the sweet potatoes being propagated by sprouts from the roots. For a period of 12 years before the specimen shown in the illustration was collected this particular strain of Haiti is known to have been propagated by sprouting of the roots. It therefore in all probability is not the result of hybridization. Inasmuch as sweet potatoes are propagated by the production of sprouts from the roots, it is practically equivalent to the perpetuation of the same plant over an indefinite period of years.

When photographic and painting records of the roots had been made, the reds and yellows were segregated and bedded and the progeny followed over a period of two years. The three red and three yellow roots were bedded in the greenhouse in the fall of 1923, and they gave in all about 25 plants of each strain. These plants were set in the field in the spring of 1924, and about one peck of potatoes were produced from each strain. The reds produced all reds, and the yellows all yellows. In the spring of 1925 all the roots of both lots were bedded, and several hundred plants of each were produced. Instead of setting out all these plants, 100 were pulled in such a manner as to obtain 2 or 3 from each potato. These were planted and cultivated in the customary way and harvested in the fall. Nearly 2 bushels of each color were obtained, the reds producing only reds and the yellows only yellows.

Figure 1, a photograph of the progenies, shows the general similarity in size and shape between the reds and yellows, there being no greater difference than would ordinarily be found between two lots of the same strain. This observation is interesting in view of the fact that the yellow potatoes of the original hill (pl. 1) are long and more slender and would ordinarily be classed as rooty. The potatoes of the Haiti variety are normally large and rather chunky. These results, therefore, would seem to indicate that the progeny tend to revert to the characteristics of size and shape of the parent stock, rather than to inherit those of a single root from an individual hill.

[1] Received for publication Dec. 19, 1925; issued September, 1926.

FIG. 1.—Progenies of the two types segregated from the plant of Haiti variety of sweet potato shown in Plate 1, showing general similarity in size and shape between the red and yellow potatoes. Propagation of the two strains was carried on for two consecutive years, the yellows (left) producing yellows, and the reds (right) producing reds



Showing color, shape, size, and arrangement of the red and yellow potatoes on the stem of the original plant of the Haiti variety of sweet potato. The yellow potatoes are more or less rooty, in contrast to the somewhat thicker red ones. (This is from a colored photograph. Potatoes were moistened before photograph was taken, to intensify the colors)

Kingsport Press, Inc., Kingsport, Tenn.

The color of the stem and vines of the Haiti variety is a deep purple. This color extends also to the petioles. In the yellow strain some of the purple pigment was lost. As a matter of fact, it has been determined from the color of the stem and vines whether a plant bore yellow or red potatoes. There has been no apparent difference in the shape, size, or color markings of the leaves, or in the time of maturity or vigor of the plants of the two strains.

9154—26——3

THE RELATION OF HUMIDITY TO INFECTION OF THE SWEET POTATO BY RHIZOPUS[1]

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INTRODUCTION

Air moisture is one of the most important environmental factors in plant responses, not only as it affects the physical conditions and physiology of the plant, but because of its influence on the infection and the development of diseases caused by microorganisms. For the most part, it has been assumed that high humidity favors, and is even essential to, infection. This generalization is based largely on observation rather than on experimental data. It seems quite likely that it is generally true, but like many generalizations it is too broad and may not hold in many cases. It has been assumed, also, that within the range of humidities at which infection occurs there is a tendency for the number of infections to increase with the increase in relative humidity. There are some data to justify this conclusion, but they are not a sufficient basis for a generalization. Neither of the assumptions just mentioned holds in connection with infection of the sweet potato by Rhizopus.

Manns (*5*),[2] who seems to have in mind the losses due to Rhizopus soft rot and black rot, regards a relative humidity of 80 per cent and above as inimical to the keeping of sweet potatoes. His conclusions were based on bell-jar and storage-house experiments. The bell-jar experiments were limited in number and there was no provision made for air exchange; and in the storage-house experiments the factors, such as temperature, humidity, and wounding, were not controlled or definitely isolated, and apparently he did not study the diseases separately. The present writers were able to confirm his results only in part.

The purpose of this paper is to present the results obtained from a study of the effects of air moisture on the infection of the sweet potato by Rhizopus (*nigricans* and *tritici*) under certain conditions.

APPARATUS AND CONTROL OF CONDITIONS

The experiments discussed below were conducted either in the infection chambers described in an earlier publication (*4*), or in a sweet-potato storage house constructed according to plans designed by the United States Department of Agriculture (*6*).

The temperature of the infection chambers was controlled in the manner described in the above-mentioned publication (*4*). The control of humidity was accomplished by means of water and saturated salt solutions in evaporation pans (*3*). Continuous air exchange

[1] Received for publication Mar. 2, 1926; issued September, 1926.
[2] Reference is made by number (italic) to "Literature cited," p. 538.

was provided in each instance (*4*). A storage house 40 feet long, 20 feet wide, and 9 feet high, was partitioned into two rooms of the same size. In the one room a high humidity was maintained by means of a fine spray of water, the small droplets of the spray being entirely evaporated in the part of the room in which the potatoes were placed. In the other room a lower humidity was obtained by means of calcium chloride in a large evaporating pan. The temperature of the two rooms was governed by the outside temperatures, and was nearly the same in both rooms in each experiment. The difference was believed to be insufficient to be a disturbing factor.

MATERIALS USED

The Yellow Jersey, Big Stem Jersey, and Porto Rico varieties of sweet potato were employed. The species of Rhizopus used were *Rhizopus nigricans* Ehr. and *R. Tritici* Saito.

EXPERIMENTAL DATA

HUMIDITY AND INFECTION OF HALVED ROOTS

The infection of the sweet potato by Rhizopus occurs almost exclusively through fresh wounds, so in order to study the influence of humidity on infection it is necessary to work with freshly wounded roots. In a series of 15 experiments the roots were cut in halves lengthwise and placed at various humidities at a constant temperature of 23° C. In 12 of the experiments the potatoes were inoculated by dipping them in a suspension of *Rhizopus nigricans* and *R. tritici* spores; in the other 3 experiments the potatoes were not inoculated. The effects of inoculation, if any, were merely to increase the percentage of infection at the various humidities. In previous work (*4*) it was found that the inoculation of wounded potatoes by means of a spore suspension did not alter the temperature range of infection of either of these species, obtained when the potatoes were not inoculated. The species isolated from decayed potatoes in these present experiments was mostly *R. nigricans*, *R. tritici* being isolated only in a few instances. This fact was in accord with previous experience (*4*). Since the absence of inoculation did not in any way alter the relations found in connection with the inoculated potatoes, the results from inoculated and uninoculated roots will be considered together.

Table 1 gives the results of seven experiments in which the Yellow Jersey was the variety of potato used. A range of five different humidities was employed in each experiment. Wherever the same humidity was obtained during the successive experiments, the results are considered collectively. The figures in the last column represent the number of times any particular humidity was employed during the seven experiments. The percentage of infecton rose from 21 per cent at humidities of 93 to 98, to 96 and 100 per cent at relative humidities of 76 to 82 per cent. At relative humidities of 48 to 53, the percentage of infection was 25 per cent. Only one experiment of this series included a relative humidity of 76, consequently the percentage of infection obtained may be high for this humidity, which will be iudicated by results recorded in Table 2.

TABLE 1.—*The relation of humidity to infection of Yellow Jersey sweet potatoes by Rhizopus*

Temperature, ° C.	Depression of wet bulb, ° C.	Relative humidity	Number of halved roots used	Number of halved roots infected	Percentage infected	Time of exposure in days	Number of experiments at each humidity
23.6	0.2–0.8	93–98	170	35	21	3–11	5
23.1	1.0–1.3	90–92	496	216	44	3–11	13
23.2	1.6–1.9	84–87	98	63	64	3–11	3
23.7	2.2–2.6	79–82	308	295	96	3–11	9
23.4	3.1	76	18	18	100	11	1
22.5	6.2–6.9	48–53	120	30	25	3–9	3

TABLE 2.—*The relation of humidity to infection of Yellow Jersey sweet potatoes by Rhizopus*

Temperature, ° C.	Depression of wet bulb, ° C.	Relative humidity	Number of halved roots used	Number of halved roots infected	Percentage infected	Time of exposure in days	Number of experiments involved
23.4	0.2–0.4	97–99	90	9	10	7–11	3
23.5	0.6–0.9	93–95	240	58	24	3–12	5
23.0	1.0–1.5	87–92	696	262	38	3–15	18
23.3	1.6–1.9	84–86	178	101	57	3–11	5
23.0	2.0–2.5	80–84	388	320	82	7–11	11
23.0	2.6–2.8	78–79	120	105	88	3–12	3
22.7	3.0–3.4	72–76	138	89	64	7–15	4
22.4	4.4–5.3	59–65	200	19	10	7–15	5
22.6	6.2–6.9	48–52	160	36	23	3–12	4

In Table 2 are recorded the results given in Table 1, combined with the results of five other experiments conducted later. These latter five experiments also had to do with the Yellow Jersey variety of sweet potato. The purpose of giving the two tables is to show the variation in the results obtained from different experiments, and at the same time to show the general consistency of the data obtained.

The percentage of infection rises from 10 per cent at relative humidities of 97 to 99, to 82 and 88 per cent at relative humidities of 78 to 84, and drops to 10 per cent at relative humidities of 59 to 65, and rises to 23 per cent at relative humidities of 48 to 52. It is not known whether this last rise has any significance, although a similar relation was found in an experiment with the Porto Rico variety. Possibly if a larger number of experiments were conducted, a higher percentage of infection at relative humidities of 59 to 65 per cent or a lower percentage of infection at relative humidities of 48 to 52 might have been obtained.

In Table 3 are given the results in which the Porto Rico variety was used. The percentage of infection increased from 15 at relative humidities of 93 to 97, to 98 at relative humidities of 75 to 76. There is a slight drop in the number of infections as the humidity is lowered. At a relative humidity of 63 the percentage of infection was 81, and at a relative humidity of 52 the percentage of infection was 75. The percentage of infection at these latter humidities is high in contrast to that obtained with Yellow Jersey. It should be noted, however, that the results were obtained from one trial at each of the two humidities from two experiments. In a third experiment run late in the season, when the outside temperature was high and consequently that of the chambers (27° C.), the percentage of infection at a relative

humidity of 61 was 22. It is possible that if a larger number of experiments were conducted at these humidities the percentage of infection might be lowered. There is a close correspondence in the percentage of infection of Porto Rico to that of Yellow Jersey at humidities between 75 and 98.

TABLE 3.—*The relation of humidity to infection of Porto Rico variety of sweet potatoes by Rhizopus*

Temperature, °C.	Depression of wet bulb, °C.	Relative humidity	Number of halved roots used	Number of halved roots infected	Percentage infected	Time of exposure in days
25	0.4–0.8	93–97	54	8	15	6–7
24	1.2–1.6	86–91	110	41	37	6–7
23.5	3.1–3.2	75–76	56	55	98	6–7
22.0	4.8	63	32	26	81	7
22.6	6.2	52	24	18	75	6

Figure 1 represents graphically the results recorded in Tables 1, 2, and 3. The percentage of infection is plotted against the depression of the wet bulb, because these figures are more exact than the relative humidities. The relative humidities are also given, so that the relation of infection to relative humidity can be seen at the same time. The correspondence of the three curves at relative humidities between 75 and 98 is striking. At the lower humidities there is considerable variation in the number of infections. It should be stated, however, that the higher humidities were obtained a larger number of times in each case than the lower humidities, thus tending to remove the effects of variation in the individual experiment. The values given in the three tables and in Figure 1 involve considerable variation and are not to be regarded as absolute, but relative. They represent general relations only.

The experiments conducted in the infection chambers were supplemented by two experiments run in a storage house partitioned into two rooms. In one room a high humidity was maintained and in the other a low. The Porto Rico and Big Stem Jersey varieties of sweet potato were used. The roots were not inoculated in either experiment. The results are recorded in Table 4.

TABLE 4.—*The relation of humidity to infection by Rhizopus, storage-house experiments*

Variety	Temperature, °C.	Depression of wet bulb, °C.	Relative humidity	Number of roots employed	Number of roots infected	Percentage infected	Time of exposure in days
Porto Rico	19.6	0.23	97	286	28	10	7
Porto Rico	28.0	0.53	96	115	33	29	5
Porto Rico	22.0	1.4	87	581	178	31	7
Porto Rico	20.0	2.6	82	125	108	86	5
Big Stem Jersey	28.0	0.53	96	173	48	28	5
Big Stem Jersey	29.0	2.6	82	98	65	66	5

The data are arranged in Table 4 for each variety of potatoes according to the humidities irrespective of the temperatures, which differed in the two experiments. In spite of this difference in temperature, there is an increase in the percentage of infection with the lowering of the humidities in the case of Porto Rico. The results of each experiment, however, should be considered separately. The first experiment had to do with Porto Rico, and had a duration of seven days. The high-humidity room was run at an average tem-

perature of 19.6° C., and the low humidity room at 22°. The average relative humidities of the two rooms were 97 and 87 per cent, respectively. The percentage of infection at a relative humidity of 97 was 10, in contrast to 31 at a relative humidity of 87.

In the second experiment both Porto Rico and Big Stem Jersey were used. The two rooms were run at an average temperature of

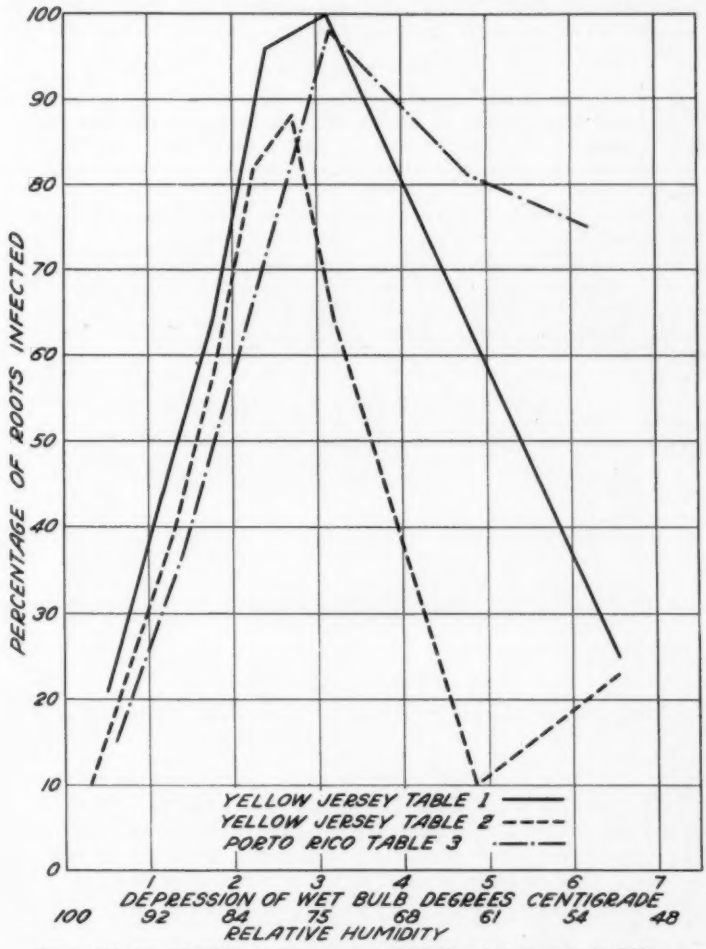

FIG. 1.—Relation of humidity to the percentage of infection of sweet potato by Rhizopus

28° and 29° C., and at an average relative humidity of 96 and 82 per cent, respectively. The experiment ran for five days. The percentage of infection of the Porto Rico at a relative humidity of 96 was 29, and 86 at a relative humidity of 82. At a relative humidity of 96 only 28 per cent of the Big Stem Jersey roots were infected, in contrast to 66 per cent at a relative humidity of 82.

The results of these two experiments within the range of humidities used correspond with the results obtained in the infection chambers, although there was not complete duplication of varieties. Yellow Jersey was not available at the time.

One experiment was conducted at a temperature of 12° C. The results, which will be published in another paper, correspond with those obtained at 23°, except that the range of infection was shifted nearer to 100 per cent humidity.

THE EFFECTS OF HUMIDITY ON HOSTS AND PATHOGENES

An effort was made to determine whether or not the relation of humidity to infection found in the foregoing experiments was due, on the one hand, to some reaction on the part of the host or, on the other, to a reaction on the part of the pathogenes, or to a combination of both.

A series of experiments were conducted in which halved roots were first subjected to one humidity for a time and then exposed to another for another interval of time at the same temperature (23° C.).

Very few potatoes (Table 5) which had first been subjected to high relative humidities (89 to 95) for from 4 to 12 days and then exposed to lower humidities (48 to 85 per cent) for 6 to 13 days became infected. If on the other hand halved roots were first exposed to low humidities (51 to 74) for 4 to 12 days and then placed at high relative humidities (84 to 95) for 6 to 31 days, a large number of infections resulted. For example, out of 48 potatoes which had been held at relative humidities of 89 to 91 and then removed to relative humidities of 80 to 85 there were no infections (Table 5). Out of 62 potatoes held at 89 to 95 and then removed to humidities of 48 to 73, 2 (or 3.2 per cent) were infected, while out of 182 potatoes held at humidities of 51 to 74 and then removed to humidities of 84 to 95, 123 (or 68 per cent) were infected.

TABLE 5.—*The effects of humidity on infection of halved roots exposed first to one humidity and then to another*

Temperature °C.	First depression of wet bulb, °C.	First relative humidity	Time of exposure in days	Second depression of wet bulb, °C.	Second relative humidity	Time of exposure in days	Number of halved roots used	Number infected	Percentage infected	Number of experiments at each humidity
23	0.4–1.3	89–97	6– 9	0.5–1.3	89–95	8–31	160	9	6	7
23	1.1–1.2	90–91	9	1.9–2.2	80–85	9	32	0	0	2
23	0.6–1.3	89–95	6–12	3.3–3.6	71–73	10–13	34	0	0	2
23	0–6–1.3	89–95	9–12	6–4–6.9	48–51	12–13	28	2	7	2
23	1.3–2.4	79–89	4–12	0.5–1.2	90–95	6–31	131	12	9	6
23	1.4	89	4	2.0	84	6	16	0	0	1
23	2.1	81	12	3.3	73	13	14	0	0	1
23	3.2–3.4	73–74	6– 7	0.5–1.1	91–95	8–31	11	3	27	2
23	4.8–5.3	59–63	6– 7	0.5–1.1	91–95	8–31	101	79	78	3
23	6.2–6.5	51–52	6–12	1.0–1.3	89–92	9–13	29	29	100	3
23	5.5–6.3	52–57	4– 9	1.9–2.0	84–85	6– 9	41	12	29	3
23	6.5	51	12	3.3	73	13	10	0	0	1
23	6.3–6.5	51–52	9–12	6.4–6.9	48–51	9–13	21	0	0	2

The potatoes held at high and low humidities were examined at the time the potatoes were changed from one humidity to another. The decayed potatoes were removed from the experiment and some of the remaining potatoes were retained at these humidities while others were removed to other humidities for a certain period (Table

5). At the end of this period very few of the potatoes retained at a high humidity (80 to 97 per cent) were infected, and none of the potatoes retained at a low humidity (48 to 73 per cent) were infected (Table 5).

Figure 2A represents the effects of exposing halved roots first to a relative humidity of 59 for 7 days, then to a humidity of 93 for a period of 11 days, at a temperature of 23° C.; figure 2B represents potatoes held at a relative humidity of 93 throughout the 18 days. The contrast is striking. Rhizopus has grown over the cut surfaces of the potatoes first exposed to a low humidity and then placed at a high humidity, whereas the potatoes exposed to the high humidity through the entire period have remained sound. At the end of the first period (7 days) the potatoes decayed at this time were removed from the experiment, so that the results represented in Figure 2

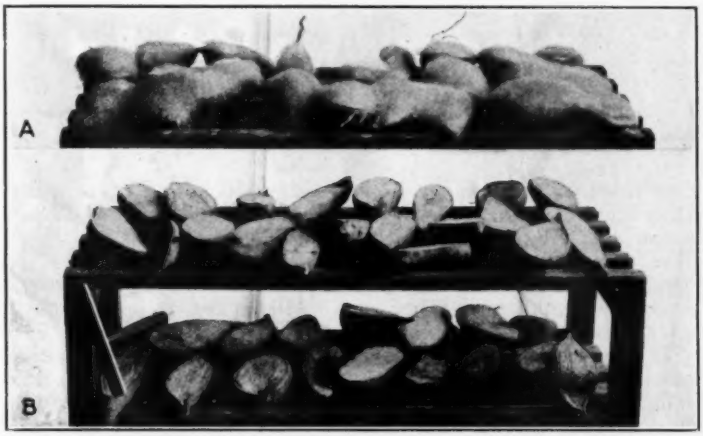

FIG. 2.—Infection of the sweet potato by Rhizopus. A, Sweet potatoes held at a relative humidity of 59 per cent for 7 days, and then at a humidity of 93 per cent for 11 days; B, Sweet potatoes held at a relative humidity of 93 per cent throughout the 18 days. Temperature 23° C. in both cases

afford a comparison of the effect of high humidity on potatoes which had previously been exposed in the one instance to a high humidity and in the other to a low humidity. It is suggested from these results that some reaction occurred in the host and not in the fungus at humidities of 85 to 100, which interfered with infection, because the fungus is capable of causing infection at high humidities if the potatoes have been subjected first to low humidities. On the other hand, very few potatoes that have been exposed to high humidities become infected no matter to what humidities they are exposed later.

It is believed that the effects of air moisture on the pathogenes interfere with infection at the low humidities, since roots that have been subjected to low humidities readily become infected when removed to high ones.

THE CHARACTER OF THE RESISTANCE EXHIBITED BY THE HOST AT HIGH HUMIDITIES

Is the resistance shown by sweet potatoes to the attack of Rhizopus at high humidities due to a reaction of the entire root, or is it located

in the wounded surface? An effort was made to answer this question in the following experiments.

Table 6 contains the results of two experiments conducted in the storage house in which Porto Rico and Big Stem Jersey varieties were used, Yellow Jersey not being available at the time.

TABLE 6.—*The effects of cutting a fresh slice from halved roots that have been exposed to high humidities*

First temperature and humidity readings			Second temperature and humidity readings			Treatment of roots	Number of roots used	Number of roots infected	Percentage infected
Temperature, ° C.	Depression of wet bulb, °C.	Relative humidity	Temperature, ° C.	Depression of wet bulb, ° C.	Relative humidity				
[a] 19.6	0.23	97	29	2.6	82	Freshly cut	40	37	93
[a] 19.6	.23	97	29	2.6	82	Old cut	39	2	5
[a] 22.2	1.4	87	29	2.6	82	Old cut	30	16	53
[b] 28	.53	96	29	2.6	82	Freshly cut	74	62	84
[b] 28	.53	96	29	2.6	82	Old cut	67	10	15

[a] These lines of figures are concerned with Porto Rico variety of potatoes.
[b] These lines of figures are concerned with Big Stem Jersey variety.

Two hundred and twenty halved roots which had been held at relative humidities of 96 and 97 per cent for a period were divided into two lots. A fresh slice, about 2 mm. thick, parallel to the cut surface, was removed from the one lot of 114 halves. The remaining 106 halves were left uncut as controls. Both lots were then placed at a relative humidity of 82 per cent. After 6 and 12 days 99 out of the 114 freshly cut potatoes (or 87 per cent) were infected, while only 12 out of the 106 (or 11 per cent) of the control potatoes became infected. Likewise 30 halved roots that had been held at a relative humidity of 87 per cent were also placed at a relative humidity of 82 per cent. Sixteen of them (or 53 per cent) became infected after 6 days. This latter percentage is high in contrast to that obtained from potatoes held first at relative humidities of 96 and 97 per cent and then placed at a relative humidity of 82 per cent, indicating that there was not as much resistance developed at a relative humidity of 87 per cent as at 96 and 97 per cent.

These results indicate that the resistance shown by sweet potatoes at high humidities is located in the surface layers of the wounded areas, because when this layer is removed the roots become infected readily when subjected to humidities that are favorable for infection. If this layer is not removed very few infections occur, even though the potatoes are placed at humidities which are favorable to infection.

Weimer and Harter (*7*) found that cork formation, in connection with wounded surfaces of sweet potatoes, took place more readily at high humidities than at low. Hauman-Merck (*2*) claims that there is some connection between cork formation and infection of sweet potatoes by *Mucor stolonifer* Ehrb. (*Rhizopus nigricans*). It is not unlikely that cork formation interferes with infection at the higher humidities, and that lack of cork formation at the lower humidities permits infection. To be certain that this relation holds it will be necessary to connect these factors more directly than has been done.

EFFECTS OF HUMIDITY ON UNWOUNDED ROOTS

Experiments were conducted during three seasons to determine whether it was possible to keep unwounded sweet potatoes at high humidities and at various temperatures throughout the season. The results are recorded in Table 7. Only 6 out of 350 potatoes held at various temperatures and at humidities ranging between 91 and 97 for 56 days during the autumn of 1921 became infected with Rhizopus. Of course, relative humidities are not comparable at different temperatures, but in any case the relative humidities were high. Table 7 gives the depression of wet bulb and humidity at each temperature so that the results can be interpreted directly.

TABLE 7.—*The relation between the exposure of Yellow Jersey sweet-potato roots to high humidities for long period and infection by Rhizopus*

Date and season	Temperature °C.	Depression of wet bulb °C.	Relative humidity	Duration of experiment in days	Number of roots used	Number of roots infected with Rhizopus	Percentage of roots infected with Rhizopus
Oct. 18, 1921–Dec. 13, 1921	28.0	1.1	92	56	50	0	
	25.5	.7	94	56	50	1	
	24.0	.8	93	56	50	0	
	20.5	.8	93	56	50	0	
	18.5	.6	95	56	50	0	
	14.0	.9	91	56	50	0	
	11.0	.3	97	56	50	5	
Total					350	6	1.7
Jan. 1, 1922–May 10, 1922	26	1.2	90	114	20	1	
	25	.9	93	114	20	1	
	24.5	.8	93	114	20	3	
	20.5	.5	95	114	20	1	
	18.0	.5	95	114	20	5	
	12.0	.3	97	114	20	4	
	11.5	.5	94	114	20	1	
Total					140	16	12
Nov. 13, 1922–Dec. 15, 1922	28	1.0	93	32	50	0	
	25	1.2	90	32	50	0	
	23	1.0	91	32	50	0	
	20	.2	98	32	50	0	
	18	.8	92	32	47	0	
	13	1.1	89	32	50	0	
	11.5	1.1	88	32	50	0	
Total					347	0	0
Nov. 13, 1922–Mar. 17, 1923	28	1.2	90	124	50	0	
	25	1.0	92	124	50	0	
	23	1.0	92	124	50	0	
	20	.2	98	124	50	0	
	18	.8	92	124	47	0	
	13	1.1	89	124	50	0	
	11.5	1.1	88	124	50	0	
	10	.5	94	124	50	1	
Total					397	1	.3
Oct. 26, 1923–Dec. 19, 1923	27	1.2	90	54	59	0	
	25	.7	94	54	74	0	
	23	.8	93	54	70	1	
	20	.7	94	54	74	0	
	18	.5	95	54	68	0	
	14	.5	94	54	79	0	
	12	.6	94	54	76	0	
	9.6	.2	97	54	77	0	
Total					577	1	.2
Oct. 26, 1923–Mar. 24, 1924	28	.5	97	149	59	8	
	25	.7	94	149	74	3	
	23	.8	93	149	70	2	
	20	.7	93	149	74	0	
	18	.5	95	149	67	0	
	14	.5	95	149	77	3	
	12	.6	94	149	73	0	
	9.6	.2	97	149	77	2	
Total					[a]571	18	3.2

[a] The discrepancy between this figure and 577, the number of potatoes used during the fall, is explained by the act that 5 potatoes were removed for other purposes.

A new lot of potatoes was used the latter part of the same season, and in this case 16 out of 140 roots (or 12 per cent) became infected. Some of the chambers in which the potatoes were stored were opened during the storage period and the temperature allowed to drop to that of the surrounding air. The humidity also dropped considerably during this period. Mice entered through the open doors in some of the chambers and gnawed some of the roots. Eight of the 16 infections took place at the point of gnawing. This fact accounts for at least 50 per cent of the infections. It is entirely possible that more of these infections may have been due to wounding caused by mice that was not macroscopically evident.

During the seasons of 1922 and 1923, 1 out of 397 roots held at constant temperatures ranging between 10° and 28° C. and at relative humidities varying from 88 to 98, became infected after 124 days.

During the season of 1923 and 1924, 19 out of 572 potatoes (or 3.2 per cent) became infected after 150 days. The temperature ranged from 9.6° to 28° C., and the relative humidities from 90 to 97 per cent.

During the three seasons, 42 out of 1,464 (or 3 per cent) became infected with Rhizopus. At least 8 of these became infected because of the gnawings of mice. It is believed that in most of the other cases of infection it was the wounding which was the chief predisposing condition for infection by Rhizopus, rather than humidity or temperature. The potatoes were handled two or three times during a season, and it is almost impossible to handle sweet potatoes without some wounding. There is nothing in the data in Table 7 to suggest that there is any relation between infection and either temperature or moisture when there is no wounding. There was not a large variation in the humidity, but the infection that did occur bore no relation to this variation. In any case, unwounded sweet potatoes can be kept at high humidities for long periods without becoming infected with Rhizopus.

DISCUSSION AND CONCLUSIONS

The optimum relative humidities for infection of sweet potatoes by Rhizopus at a temperature of 23° C. range between 75 and 83 per cent. The percentage of infection decreases rapidly as the humidity is raised above 83 per cent or lowered below 75. The decrease is more consistent above 83 per cent than below 75. This consistency may have some relation to the number of times the higher humidities were attained in these experiments in contrast with the lower humidities. Whether there is some disturbing factor at the lower humidities that may account for this irregularity in results is not known.

The consistency of the results, whether considered in connection with the individual experiment or the experiments as a whole, is striking, when it is understood that the percentage of infection is used as the measure of the effects of humidity.

The relation of humidity to infection is similar in the case of the Yellow Jersey and Porto Rico varieties. Big Stem Jersey responded in a similar fashion to the humidities to which it was exposed.

The relation between humidity and infection of sweet potatoes by Rhizopus would seem to be governed by reactions on the part of the host and the pathogenes. Why is it that sweet potatoes become infected with Rhizopus less readily at humidities above 90 per cent

than at humidities of 75 to 85? Is it because the higher humidities are less favorable to the pathogenes? This is believed not to be the case, for if it were, why do potatoes that have been held at the lower humidities become infected when removed to humidities of 90 and above? It is believed, rather, that some change occurs in the host that renders it immune, because potatoes that have been held at high humidities become infected but rarely when subjected to lower humidities.

In what does this resistance consist? We have a hint from Weimer and Harter's work, "Wound-Cork Formation in the Sweet Potato" (*7*). They found that cork formation in connection with wounded surfaces occurs more readily at high humidities than at low. The concomitant relation of infection and cork formation to humidity would seem to have some significance, particularly since the resistance exhibited by roots at high humidities is located in the wounded surface. However, further work is required to determine more definitely the relation of cork formation to infection.

Why is it that infection does not take place as readily at relative humidities below 74 per cent as it does at humidities between 75 and 83? This question can not be definitely answered at present. It is believed that it is because moisture becomes a limiting factor to the pathogenes; i. e., these low humidities do not furnish sufficient moisture for the germination of the spores and the penetration of the germ tubes. Of course it is possible that the host may resist infection at these humidities, but it seems unlikely, because these same roots become infected when they are placed at high humidities. At any rate, if the absence of infection is due to the condition of the host, other than the lack of sufficient moisture for the pathogenes, this condition is transitory.

These results may explain the inability of Harter, Weimer, and Adams (*1*) to obtain infection of sweet potatoes by Rhizopus in moist chambers. The humidity in the moist chambers was too high to permit of infection. By use of the "well" method of inoculation they were able to obtain infection in moist chambers. The success of the "well" method of inoculation, may possibly be explained on the basis that the process of infection was thus hastened because of the amount of inoculum used, thereby preventing the development of the condition which prevents infection at high humidities. It is possible by other means (*4*) to prevent the development of the resistance at high humidities sufficiently to permit of infection. If the roots are severely wounded by striking them over the edge of a wire basket they become infected readily at high humidities. This fact may possibly be explained by Weimer and Harter's work on cork formation in wounded surfaces. They found (*7*) that when roots were wounded by striking them over the edge of a wire basket, cork formation did not take place at the bottom of the wounds. Such interruption of this process would account for infection in deep and severe wounds at high humidities, even though cork formation normally occurs in an atmosphere of high moisture content.

The success in keeping unwounded sweet potatoes at high humidities throughout the season without their becoming infected can be explained on the basis that Rhizopus rarely infects sweet potatoes except through fresh wounds.

It is possible that if freshly dug potatoes—which of course are freshly wounded—were placed at low humidities, they might become infected if they were exposed to high humidities later. Experiments are now under way to determine whether this is true.

Weimer and Harter's work (7) suggests that excessive drying of freshly dug sweet potatoes renders them more susceptible to the attack by Rhizopus. How often sweet potatoes are excessively dried during the process of curing is not known.

SUMMARY

The data recorded in this paper, and those obtained from other experiments, show that infection of sweet potatoes by Rhizopus occurs almost exclusively through fresh wounds.

The optimum relative humidities at a temperature of 23° C. for the infection of halved roots of sweet potatoes by Rhizopus range from 75 to 84 per cent.

The percentage of infection decreases rapidly as the humidity is raised above or lowered below the optimum humidities.

This decrease is more consistent above the optimum than below it; i. e., there is less variation in the number of infections above the optimum than below it.

Very few infections of halved roots occurred at relative humidities of 93 to 99 per cent at a temperature of 23° C.

Very few halved roots which had been subjected to relative humidities of 89 to 97 per cent (temperature 23° C.) for a period of 4 to 12 days became infected when they were retained at these humidities for another period or were placed at humidities of 48 to 89 per cent.

On the other hand, a high percentage of the halved roots which were subjected to relative humidities of 51 to 73 per cent for a period of 4 to 12 days became infected when they were placed later at relative humidities of 84 to 95 per cent.

Halved roots that had been exposed to relative humidities of 51 to 52 per cent for a period of 9 to 12 days did not become infected when they were subsequently placed at relative humidities of 48 to 73 per cent.

A resistance to infection by Rhizopus developed in the halved roots that had been held at relative humidities of 89 to 97 per cent.

This resistance is located in the surface layers of the wounded areas and has a possible relation to cork formation.

The Yellow Jersey, Big Stem Jersey, and the Porto Rico varieties of sweet potato responded in a similar fashion (within the range of humidities used in each case) to the action of air moisture.

The Yellow Jersey variety was kept for periods of 56 to 150 days during three seasons with only a few infections with Rhizopus occurring.

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THE USE OF SODIUM NITRITE IN THE CURING OF MEAT[1]

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INTRODUCTION

The art of preserving meats by curing in common salt, with or without smoking, is one which has been practiced from remote antiquity. Directions for curing meats with salt appear in ancient writings, and the art is still practiced among primitive peoples. Yet, notwithstanding the antiquity of the art and its economic importance, adequate and comprehensive scientific study with publication of results has never been given to it. Even at the present time the scientific publications on the subject consist largely of recipes and formulas or the results of desultory and isolated experiments.

The use of nitrates also dates from ancient times, and has been practiced so long that its origin is unknown (*2, p. 613*)[2]. Only within recent years, however, has the function of nitrates been correctly understood. The investigations of Haldane (*1*) and Hoagland (*3, 4*) demonstrated that this function is the fixation of color which takes place through the reduction of nitrate to nitrite, and the combination of the latter with the hemoglobin of the meat. McBryde (*7*) showed that potassium nitrate has no more preservative power than common salt when tested against the organisms concerned in the spoilage of hams. Present scientific knowledge, therefore, indicates that nitrates are useful only as color fixatives and that their aid in preservation is not of importance.

The nitrates, as such, are without value as color fixatives and become active only when reduced to nitrites. In practice this is proved by the fact that if the formation of nitrites is prevented or interfered with in any way the color fixation is likewise prevented or interfered with. Unsatisfactory or insufficient formation of nitrite invariably results in unsatisfactory, inadequate, or irregular fixation of color. So far as color fixation is concerned, the progress of curing in a vat of meat in pickle may be followed by determining the nitrite content of the pickle.

The formation of nitrite was ascribed by Haldane (*1*) to bacterial action. This opinion is evidently correct, as there are several circumstances connected with practical meat curing which are best explained on the assumption that the nitrate is reduced by bacterial action. Among these are the variability of the cure, often observed in a new establishment, the advantageous results obtainable by adjustment and control of the hydrogen-ion concentration of the pickle, the benefits derived from "seeding," and the occasional failure of nitrite formation caused by development of acid-forming organisms in the pickle.

[1] Received for publication Apr. 20, 1926; issued September, 1926.
[2] Reference is made by number (italic) to "Literature cited," p. 551.

The source of the nitrate-reducing organisms has never been definitely ascertained, but in all probability it is multiple. Nitrate-reducing organisms are so widely distributed in nature that it would be difficult to find an environment in which they are not present. From general knowledge of the occurrence of such organisms it is to be presumed that the water used in making up the pickling solutions, the tables, trucks, and other equipment employed in handling the meats, the curing vats and boxes, and the meats themselves, are sources of nitrate-reducing organisms. It has been observed that organisms capable of effecting the conversion of nitrate to nitrite in the presence of such concentrations of salt as are commonly used in meat curing can be readily isolated from the fresh pickling solutions. Considerable variability in the vigor of growth and the rapidity and completeness of the conversion effected has been observed among different isolations. Other observations indicate that the curing vats are constant and reliable sources of the active nitrate-reducing organisms. Although it is customary to steam out the vats after use, it is known that this steaming does not result in sterilization, and a vat once well inoculated with a vigorous and effective nitrate-reducing organism would doubtless harbor the organism for an indefinite and extended period. The uniformity of curing action which is often attained and maintained for long periods is explainable on this basis.

The curing action proceeds, as a rule, with remarkable uniformity and regularity. An example of the typical course of nitrite formation is given in Table 1.

TABLE 1.—*Changes in nitrite content of nitrate pickle*

Day	$NaNO_2$ (parts per million)	Day	$NaNO_2$ (parts per million)
1st	3.6	41st	421.0
5th	10.0	48th	540.0
15th	33.0	55th	700.0
26th	138.0	62d	820.0
30th	189.0	65th	887.0

In contrast to the typical and satisfactory course of nitrite formation cited above, various degrees of unsatisfactory formation, ranging from delayed formation necessitating a brief prolongation of the curing period to nearly total failure of nitrite formation, have been observed.

Any deviation from the normal process is, of course, a source of loss, inconvenience, and expense. Means of eliminating the irregularities of nitrate formation and obtaining uniformity of curing have naturally been much sought. The means employed and the results obtained have not been published, but have been held as trade secrets by the various establishments using them. Consideration of the problem indicates that it may be solved either by the cultivation of a suitable nitrate-reducing organism in the pickle or by the direct addition of the required nitrite as such instead of depending on the formation by bacterial action. The first method, while unquestionably feasible, has the disadvantage of requiring expensive supervision, and is not applicable in the absence of a scientific laboratory. This method is therefore open only to the larger establishments. No ad-

vantage over the direct use of nitrite either from the standpoint of operations or of public health is apparent. The direct use of nitrite is therefore suggested as the method of choice. The experiments described herein were undertaken in order to determine the practicability of using nitrite directly and the advantages and disadvantages connected with such use. On the part of the bureau the possibility of harm to human health was of course given primary consideration.

EXPERIMENTS IN DIRECT USE OF NITRITE

The first experiment involving the direct use of nitrite was formally authorized January 19, 1923, as a result of an application by one of the large establishments operating under Federal meat inspection. Before that time other requests for permission to experiment with nitrite had been received but had not been granted. The authorization for the first experiment specified that the whole process was to be conducted under the supervision of bureau inspectors and that after the curing had been completed the meat was to be held subject to laboratory examination and final judgment and would be destroyed if found to contain an excessive quantity of nitrites or if in any way it was unwholesome or unfit for food. This principle was rigidly adhered to throughout the experimental period, no meat being passed for food until its freedom from excessive nitrites had been assured, either by laboratory examination or through definite knowledge from previous examinations, that the amount of nitrite used in the process would not lead to the presence of an excessive quantity of nitrites in the meat.

By "excessive" is meant a quantity of nitrite materially in excess of that which may be expected to be present in similar meats cured by the usual process. The analyses given in Table 2 are typical of the nitrite content of meats cured with nitrate in accordance with the customary practice.

TABLE 2.—*Nitrite content of meats cured by nitrate process*

Kind of meat	$NaNO_2$ (parts per million)	Kind of meat	$NaNO_2$ (parts per million)
Ham (whole slice, ground)	72.0	Ham	60.0
Do	40.0	Ham (outside portion of piece)	480.0
Do	88.0	Ham (inside portion of piece)	192.0
Do	104.0	Ham (outside portion of piece)	624.0
Corned beef	3.6	Ham (inside portion of piece)	288.0
Do	2.4	Ham (outside portion of piece)	576.0
Do	41.0	Ham (inside portion of piece)	480.0
Do	24.0	Ham (outside portion of piece)	960.0
Do	12.0	Ham (inside portion of piece)	576.0
Do	12.0	Bacon (outside portion of piece)	120.0
Salt Pork	7.2	Bacon (inside portion of piece)	104.0
Do	2.4	Bacon (outside portion of piece)	640.0
Dried beef	7.2	Bacon (inside portion of piece)	624.0
Do	12.0	Bacon (outside portion of piece)	144.0
Boiled ham	12.0	Bacon (inside portion of piece)	72.0
Do	17.0	Bacon (outside portion of piece)	120.0
Do	24.0	Bacon (inside portion of piece)	96.0
Salt pork	2.4	Bacon (outside portion of piece)	96.0
Do	208.0	Bacon (inside portion of piece)	56.0
Do	14.4	Bacon (outside portion of piece)	336.0
Do	240.0	Bacon (inside portion of piece)	288.0
Smoked shoulder	180.0	Dry-salt butt	1.5
Salt pork	2.4	Do	2.9
Ham	60.0	Dry-salt fatback	1.9
Corned beef	60.0	Do	1.5
Ham	48.0	Boiled ham	16.0
Do	36.0	Smoked ham	568.0

Under the terms of the initial authorization, 92 pieces of meat weighing in all 1,142 pounds were cured in pickle containing approximately 0.2 per cent of sodium nitrite, and 94 pieces of bacon, weighing 1,270 pounds, were cured in boxes with a dry mixture containing sodium nitrite. These experiments were paralleled by like quantities of meat cured in pickle and with a dry mixture containing the usual proportions of sodium nitrate. Analyses were made of the finished meats, as well as of the curing materials used, and of the pickles at different ages. The finished meats were also tested by cooking and serving to a considerable number of persons, of whom some knew that an unusual process of curing had been used, and some did not. The judges were all department employees, and included trained scientists, experienced meat inspectors, and others familiar with commercially cured meats.

Close attention was given to the quality and flavor of the finished meats as well as to nitrite content throughout the experiments, since it is obvious that any change in the customary curing process which resulted in impairing the quality of the product would not be in the public interest. The general plan outlined above was followed throughout the experimental period.

The changes in the nitrite content of the pickle in two vats of hams in nitrite cure are shown (Table 3) for comparison with the corresponding changes in a typical nitrate cure (Table 1).

TABLE 3.—*Changes in nitrite content of nitrite pickle*

Day	$NaNO_2$ (parts per million) in—		Day	$NaNO_2$ (parts per million) in—	
	Vat A	Vat B		Vat A	Vat B
1st	1,820	1,820	48th	864	824
5th	1,365	1,365	55th	850	790
15th	1,259	1,213	62d	810	720
26th	1,100	1,040	65th	730	----
30th	1,058	1,024	68th	----	720
41st	8.6	830			

An examination of five of the hams cured with sodium nitrite showed an average nitrite content ranging from 42 to 150 parts per million. The nitrite content was found to be greatest in that part of the ham nearest to the flesh side and least in the center next to the bone. The maximum nitrite content of any part of any nitrite-cured ham was 200 parts per million. The hams cured with nitrate in the parallel experiment showed a maximum nitrite content of 45 parts per million.

From the standpoint of flavor and quality the results were satisfactory. All judges agreed that the nitrite-cured meat was fully equal in quality and flavor to that cured by the customary process. No deficiency in quality or flavor was noted by any of them, and none of them were able to distinguish the nitrite-cured meat from that cured by the customary process.

In addition to the curing of hams in sweet pickle, the initial experiment covered the curing of bacon, both in sweet pickle and by the box process. The results with both types of bacon were

also successful. The initial experiment therefore demonstrated that hams and bacon could be successfully cured with sodium nitrite, and that nitrite curing need not involve the presence of as large quantities of nitrite in the product as sometimes are found in nitrate-cured meats.

Following the successful outcome of the initial experiment, further experiments were authorized, first at the original establishment and subsequently at others. In all, experiments were conducted at 17 establishments in 13 cities. These establishments ranged in size from two of the largest in operation to those of rather small size, and included some at larger centers of consumption as well as several in the producing sections. The products cured included pork shoulders, shoulder butts, pork loins, pork trimmings, pork tongues, beef tongues, lamb tongues, corned beef, and dried beef, as well as hams and bacon. Representatives of the Institute of American Meat Packers participated in the experiments at certain establishments. Their results have been published elsewhere (*5*, *6*). The duration of the experiments ranged from two years and eight months at the first establishment to a few weeks at the last establishment permitted to conduct experiments. The quantity of meat cured with sodium nitrite was large, as all establishments were permitted to make their experiments on a commercial scale. The results published here were therefore derived from full-scale operations and are directly applicable to commercial meat curing.

Supervision of the experiments followed the plan outlined in the first experiment. No control was exercised over the curing formulas except that a rather liberal limit was set to the quantity of nitrite which might be used and the establishment was not permitted to exceed that limit. The first experiment having shown it to be easily attainable, 200 parts per million was fixed as a tentative limit for the nitrite content of the finished meats.

Results of large-scale operations confirmed those of the first experiment. It was demonstrated at each establishment which conducted experiments that sodium nitrite could be successfully substituted for the sodium or potassium nitrate customarily used, and that the direct use of nitrites did not involve any increase in nitrite in the finished meat. Results for each establishment will not be given, since this would involve a needless repetition.

All operations were conducted under the direct supervision of Federal inspectors. Packing-house officers and employees, however, arranged for and conducted the actual handling of the meat.[3]

The results obtained with hams in sweet-pickle cure at two establishments at which nitrite-curing experiments were conducted on a considerable scale are given in Table 4.

[3] The writers acknowledge the excellent cooperation of the many Federal inspectors and packing-house employees who participated in this work and who furnished the data on which the results here reported are based.

TABLE 4.—*Sodium nitrite in corresponding samples of pickle and of meat*

Establishment A. $NaNO_2$ (parts per million)		Establishment B. $NaNO_2$ (parts per million)	
Pickle	Meat	Pickle	Meat
500	20	450	60
450	20	600	30
630	30	500	50
650	35	650	70
625	50	600	50
550	40	500	70
600	35	650	70
600	50	600	70
600	40	650	60
550	30	550	60
500	40	450	40
400	30	650	100
450	20	550	60
400	80	600	80
375	30	400	70
450	80	650	70
450	50	550	80
400	90	450	90
550	95	600	85
600	90	550	70
450	100	600	90
550	60	550	90
550	65	600	85

The first column shows the nitrite strength of the pickle at the conclusion of the curing period. The second shows the nitrite content of the meat cured in the pickle represented by the corresponding sample. The nitrite content of the pickle at the conclusion of the curing period ranges from 375 to 650 parts per million. This corresponded to a nitrite content of from 20 to 100 parts per million in the smoked hams. These samples were collected at approximately monthly intervals during a period of 18 months and show results of large-scale operations.

Comparable results were obtained on the other products cured with sodium nitrite. In no case and in no establishment did the nitrite content of the finished product exceed, or regularly approach, the tentative limit of 200 parts per million, after the employees of the establishment had become familiar with the use of nitrite.

Some differences were noted in the application of nitrite curing to individual products, although the underlying principles, of course, are applicable to all products. Curing in all cases and in all products consists in the absorption of sufficient salt to preserve the meat, the fixation of color, and the development of that distinctive flavor and texture which distinguishes cured from fresh meats. If any one of these three factors fails the results are unsatisfactory. Differences in curing methods for different products are determined by the size, shape, and character of the different cuts and products and to a less degree by the salt content and flavor desired in the finished product.

The irreducible minimum of time in all curing is that period required for the meat to take up the required quantity of salt. In curing with nitrates the absorption of salt runs ahead of the fixation of color, since salt penetration begins as soon as the meat is placed in cure, while color fixation can not begin until nitrite is formed. In nitrate curing, therefore, the meats must be held in cure for a more or less extended period after they have taken up the necessary salt. By the use of nitrite this additional period of waiting for color fixation

is avoided, as color fixation, like salt penetration, begins at once. For this reason the use of nitrite makes possible a material shortening of the curing period. In actual practice, with one exception, a shortening of the curing period was realized at all of the establishments in which experiments were conducted. The exception was an establishment in a large consuming center, which had been using an exceptionally rapid curing process. Although no reduction of the curing period was accomplished in this instance, the quality of the product was materially improved. In one establishment the shortening amounted to 60 per cent of the former curing period; in the other establishments the shortening amounted to from 10 to 40 per cent of the former curing period.

The flavor and quality of the meat cured with nitrite was in all cases at least equal to that cured with nitrate by the process regularly used by the establishment. In one case no improvement could be detected, the nitrite-cured meat being identical with that cured by the establishment in the regular way. In all other cases an improvement in quality was discernible after the nitrite-curing process had been established on a working basis.

The quantity of sodium nitrite required for successful fixation of color varies according to the process employed. One-fourth of an ounce, or less, of sodium nitrite appears to be sufficient to fix the color of 100 pounds of meat. In case of chopped meats, such as trimmings, where all the curing materials added remain in the meat and none is lost in the form of unused pickle or residual liquid, this proportion has been found to be sufficient. In other products the use of somewhat more nitrite is required. In any case 1 ounce of sodium nitrite appears to be sufficient for 100 pounds of meat. The minimum quantity used in any experiment in the curing of hams in sweet pickle corresponded to 6⅔ ounces of sodium nitrite to 100 gallons of pickle, or approximately ⅓ ounce to 100 pounds of meat. This proportion was insufficient for satisfactory color fixation, as also was a pickle containing 13⅓ ounces in 100 gallons, or approximately ¾ ounce to 100 pounds of meat. On the other hand, a pickle containing ½ pound of sodium nitrite in 100 gallons, or a little less than ½ ounce to 100 pounds of meat, was used with success at another establishment. In the latter case, however, the hams were pumped with a stronger pickle, so that the quantity of nitrite actually added to the meat is not accurately known and was more than that contained in the pickle. From the information available it appears that the nitrite strength of either curing or pumping pickles should not be less than ½ pound or more than 2 pounds to 100 gallons. This applies to all products cured in pickle, either plain or sweet; that is, with or without sugar.

At one establishment hams cured in a nitrite pickle were also pumped with a pickle containing a large quantity of nitrate. The pumping pickle was also of high salt strength. The nitrate injected with the pump pickle was found to remain in the ham without material change throughout the curing process, so that the hams at the completion of the cure contained only such nitrites as were derived from the curing pickle. After cutting and exposure of the cut surfaces to the air, rapid reduction of nitrate took place, so that within a short time the meat contained large quantities of nitrites. At the

same time a decidedly disagreeable flavor was developed. After applying the obvious remedy, namely, replacement of the nitrate-pumping pickle with one containing a suitable proportion of nitrite, further results were satisfactory.

At several other establishments nitrite and nitrate were tried in combination. No advantage was found to result from the combination in any case. If nitrite is used at all, it appears desirable to employ a sufficient amount to fix the color of the meat. If this is done, no advantage from the addition of nitrate is evident.

Several establishments used nitrite in the curing of bacon by the box process. This process consists in packing the bacon in a water-tight box with the curing mixture. Although often referred to as a dry-curing process, it is not that in fact, as the curing mixture within a very short time forms a solution through extraction of fluid from the meat, and the meat is thereafter immersed in the liquid thus formed. The process is therefore "dry" only in the sense that no fluid is added. The proportions of nitrite used ranged from ¼ ounce to 1¼ ounces to each 100 pounds of meat. All proportions used were successful, the smallest being sufficient for satisfactory color fixation while the greatest did not result in excessive nitrite in the meat. The experiments demonstrated the necessity of thorough mixing of the curing ingredients, as in one instance the meat was damaged by local action of nitrite resulting from imperfect mixing. Each establishment which experimented with this process accomplished a material shortening of the curing period.

In curing pork loins with nitrite one establishment failed to obtain a satisfactory color and attempted to get better results by increasing the proportion of nitrite. The attempt proved unsuccessful, and it was found that the failure was caused by a lack of hemoglobin in the meat rather than of nitrite in the curing mixture. In this instance ¼ ounce of sodium nitrite was found to be more than sufficient to secure the maximum possible color in 100 pounds of pork loins. The results of this experiment are of particular interest in showing that a lack of color in the product may be caused by a lack of sufficient hemoglobin rather than by a lack of nitrite, and that when such is the case it is of no avail to increase the proportion of nitrite.

Experiments involving the use of nitrite in smoked and cooked sausage were of particular interest. In the manufacture of sausage of this type it is customary to use a curing process. In this process all or part of the trimmings may be cured before chopping, or the curing agents may be added to the chopped meats and the mixture allowed to stand long enough, under suitable conditions, to attain the effect desired. In either case the use of nitrate is customary and the formation of nitrite and fixation of color are essential parts of the curing process. The curing period being in all cases short, the proportion of nitrate commonly used is liberal. This may result in the presence of considerably more nitrite than is required in the finished product. In Table 5 are given the results of a survey which included all brands of sausage of the Frankfurter style and imitation sausage resembling Frankfurter style prepared at establishments operating under Federal inspection at one meat-inspection station of the bureau.

TABLE 5.—*Nitrite content of Frankfurter style sausage and imitations prepared with nitrate*

Sample No.	Establishment	Product	$NaNO_2$ (parts per million)
5475	A	Imitation Frankfurter	Trace.
5476	A	Frankfurter style sausage	30
5578	B	Imitation Frankfurter	160
5580	B	Frankfurter style sausage	160
5581	B	do	160
5582	B	do	250
5583	B	do	625
5642	C	Imitation Frankfurter	20
5643	D	Frankfurter style sausage	1,400
5644	D	Imitation Frankfurter	50
5645	C	Frankfurter style sausage	20
5682	E	Imitation Frankfurter	140
5683	E	Frankfurter style sausage	500
5684	E	do	250

Nitrate was used in the preparation of the products represented by all of the samples shown in Table 5. The quantities of nitrate were such as are commonly employed, and no unusual process of curing or manufacture was used. The results, therefore, are typical of what may be expected from the ordinary and usual processes of sausage manufacture. An examination of a large number of samples collected from numerous establishments in different cities has shown that the variations in nitrite content indicated in Table 5 are typical and not exceptional.

The experiments involving the use of nitrite in sausage showed that the curing period can be materially shortened. The nitrite being added in the form of the pure salt, the quantity can be accurately controlled and the variation in the nitrite content of the product prevented. It was found that one-fourth ounce or less of sodium nitrite is sufficient to fix the color in 100 pounds of sausage meat. The results shown in Table 6 are typical of the nitrite content of sausage prepared with sodium nitrite.

TABLE 6.—*Nitrite content of sausage prepared with nitrite*

Sample No.	Establishment	Product	$NaNO_2$ (parts per million)
1211	E	Frankfurter style sausage	40
4527	F	Bologna style sausage	60
2886	G	Frankfurter style sausage	150
2898	G	Luncheon meat	80
3627	G	Minced luncheon	50
3628	G	Frankfurter style sausage	50
3646	G	do	60

It is evident from Table 6 that the nitrite content of sausage can be controlled within very much narrower limits when nitrite is used instead of nitrates.

EFFECT OF THE USE OF NITRITE ON THE PUBLIC HEALTH

During the whole course of the experiments the question of possible harm or benefit to the health of the public was given primary consideration. The presence of nitrites in cured meats, however, was already sanctioned by the authoritative interpretation of the meat inspection and pure food and drugs acts sanctioning the use of saltpeter (*9*); as shown previously, meats cured with saltpeter and sodium nitrate regularly contain nitrites. The questions to be decided, then, were whether sodium nitrite could be successfully substituted for saltpeter or sodium nitrate, whether the residual nitrite in the meats cured with sodium nitrite would be greater than that in meats cured in the usual manner, and whether the meats cured with sodium nitrite would in any way be less wholesome than those cured by the usual process. The results have shown that nitrites can be successfully substituted for nitrates. No curing difficulties caused by the use of nitrites were observed. At no establishment was any increase in the proportion of spoilage observed. The residual nitrites found in the nitrite-cured meats were less than are commonly present in nitrate-cured meats. The maximum quantity of nitrite found in nitrite-cured meats in particular was much smaller than the maximum resulting from the use of nitrate. The nitrite-cured meats were also free from the residual nitrate which is commonly present in nitrate-cured meats. No other condition or character was observed in the nitrite-cured meats which could possibly make them any less wholesome than if cured with nitrate. No objection to the substitution of nitrite for nitrate from the standpoint of public health was therefore disclosed by the experiments. On the contrary, the more accurate control of the amount of nitrite and the elimination of the residual or unconverted nitrate are definite advantages attained by the substitution.

SUMMARY AND CONCLUSIONS

Sodium nitrite can be successfully substituted for sodium or potassium nitrates in the curing of meat.

From one-fourth to 1 ounce of sodium nitrite is sufficient to fix the color in 100 pounds of meat, the exact quantity depending on the meat to be cured and the process to be employed.

Meats cured with sodium nitrite need contain no more nitrites than meats cured with nitrates, and are free from the unconverted nitrates regularly present in nitrate-cured meats.

A shortening of the customary curing period may be obtained by the use of nitrite.

Meats cured with sodium nitrite in the proper quantity and in accordance with sound practice are in no way inferior in quality or wholesomeness to meats cured with nitrates.

As a result of the experiments described above, the use of sodium nitrite in meat curing in Federally inspected establishments has been formally authorized by the Department of Agriculture (*8*), subject to meat-inspection regulations.

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ELECTRODIALYSIS OF THE COLLOIDAL SOIL MATERIAL AND THE EXCHANGEABLE BASES[1]

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INTRODUCTION

Recent investigations in the Bureau of Soils have shown that the colloidal material present in any soil contains 10 or more elements in widely varying proportions (*19*).[2] Various attempts to fractionate the material into substances of more definite composition have not met with much success. However, dialysis of the material indicated that one colloid held about 1 per cent of its constituents less firmly than the remainder. Long-continued treatment apparently removed all the lime, soda, and sulphur but only a small part of the magnesia, potash, and other elements.

It was thought that further evidence concerning the firmness with which the different constituents are held in the colloid might be obtained by electrodialyzing the material. Electrodialysis is similar to ordinary dialysis, but is much more rapid and effects a separation of the diffusible ions of opposite charge.

The mass of data that has been accumulated on base exchange in soils indicates that the colloidal soil material must contain a fairly definite part of its bases in a reactive condition. It was therefore important to compare the bases that can be removed by electrodialysis with the quantity removable by base exchange with a salt solution.

PREVIOUS WORK

While electrodialysis has been used in other fields of investigation, only a few attempts appear to have been made to apply it to the study of soils. Cameron and Bell (*4*) studied the results of continuously removing the products of hydrolysis from powdered hornblende, apatite, etc., by the use of the electric current. Gradually decreasing, small amounts of monovalent and divalent bases were thus removed. It is therefore evident that the minerals of a soil would be attacked to a slight extent by this treatment.

König, Hasenbäumer, and Hassler (*15*) and later König, Hasenbäumer, and Kuppe (*16*) studied the amount of material removable from different soils by the electric current and found that there was some correspondence between the material removed by electrodialysis and that removed by steam and hydrogen peroxide.

The study of base exchange in soils, which dates back to Way (*20*), has lately received new impetus through the work of Gedroiz (*8*) in

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[2] Reference is made by number (italic) to "Literature cited," p. 566.

Russia, Kappen (*17*) in Germany, Hissink (*13*) in Holland, and Kelley and Brown (*14*) in this country. The following facts established in numerous investigations bear on the subject dealt with in this paper. It has repeatedly been shown that in place of the bases removed from a soil by treating it with a neutral salt solution an approximately equivalent quantity of the cation of the salt is adsorbed. As to the quantity of exchange, it has been shown that while this varies in any single treatment with the concentration and volume of the solution and with the kind of cation, approximately the same end point is reached when different salts and concentrations are used, provided the soil is treated a sufficient number of times. This indicates that a given soil contains a fairly definite quantity of bases present in a particularly reactive condition.

These facts were established for the whole soil, but it was generally believed that the reactive bases were present in the colloidal material and this is now known to be so. A recent study (*1*) shows that the adsorbing power of a soil for various substances is practically all due to the colloidal fraction, and recent work of Kelley and Brown (*14*) indicates that this is also true in the case of exchange adsorption with inorganic salts.

Since practically all the investigations of base exchange were conducted on whole soils in which neither the quantity nor composition of the colloid was known, no data are available to show what part of the total bases in the colloid are exchangeable.

METHODS

For the following work colloidal fractions of the Sharkey clay soil and the Norfolk fine sandy loam,[3] prepared by the supercentrifuge method as explained in a previous publication (*10, p. 16*), were selected. These two colloids in chemical composition and colloidal properties are representative of the wide differences that have been encountered in the examination of a large number of soil colloids (*19*). The Sharkey colloid is gray in color, has a high $\frac{SiO_2}{Al_2O_3 + Fe_2O_3}$ ratio, a high content of monovalent and divalent bases, a high adsorptive capacity for ammonia gas and basic dyes, and is strongly electronegative as measured by the amount of methylene blue required to render a given quantity of the clay isoelectric. The Norfolk colloid is yellow, has a low $\frac{SiO_2}{Al_2O_3 + Fe_2O_3}$ ratio, a low content of monovalent and divalent bases, a low adsorptive capacity, and is weakly electronegative.

Table 1 gives the composition of these two soil colloids essentially as determined by the fusion method of the Association of Official Agricultural Chemists (*3*). Carbonates were absent in both samples, and easily soluble salts, which were low in the original soils, were further reduced by the large quantities of distilled water used in the separation of the colloidal material.

[3] The sample of Sharkey clay soil was taken to a depth of 4 inches, near Valley Park, Issaquena County, Miss. The sample of Norfolk fine sandy loam was taken from 12 to 36 inches, one-fourth mile south of Scottsville, Wayne County, N. C.

TABLE 1.—*Analysis of the Sharkey clay and Norfolk fine sandy loam soil colloids*

Kind of colloid	SiO_2	TiO_2	Al_2O_3	Fe_2O_3	MnO	CaO	MgO	K_2O
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Sharkey	52.05	0.51	22.52	8.12	0.035	1.36	2.52	1.89
Norfolk	39.25	.82	33.61	11.24	.005	.30	.44	.49

Kind of colloid	Na_2O	P_2O_5	SO_3	Ignition	Total	Organic	Mols. SiO_2 / Mols. Al_2O_3 & Fe_2O_3
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	
Sharkey	0.21	0.64	0.20	9.99	100.05	3.92	3.18
Norfolk	.25	.23	.05	14.04	100.73	1.90	1.63

The exchangeable bases in the colloid were determined by leaching with normal NH_4Cl, essentially as proposed by Hissink (*13*) for soils, and also by treating with 0.05 normal HCl, as recently proposed by Gedroiz (*9*). Briefly, the procedure for extraction with NH_4Cl was as follows: A 10 gm. sample of the colloid was shaken up with an ammonia chloride solution and then leached with 1 liter of hot normal NH_4Cl. The bases were determined in the filtrate. The procedure for extraction with 0.05 normal HCl was similar, except that 5 gm. of colloid were used and 300 c. c. of acid.

The apparatus used for electrodialysis was a three-chambered cell. The central chamber containing the soil material was separated from the outer electrode chambers by means of parchment paper. The cell was constructed by bolting together three U-shaped pieces of soft plate rubber (B_1, C, B_2) and two sheets of parchment paper between two hard rubber plates (A_1 A_2) as shown in Figure 1. The electrodes (E_1 E_2) were of platinum wire gauze and were large enough to extend to the sides and bottom of the chambers. A lattice of glass rods, not shown in the figure, was placed in each of the electrode chambers to support the parchment and prevent bulging when the central compartment was filled with the soil material. The electrode chambers were provided with outlets (O_1 O_2)[4] to facilitate the withdrawal of the electrodialysates.

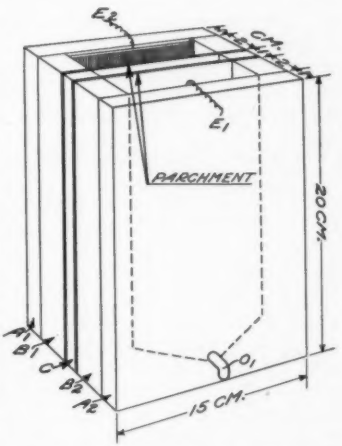

FIG. 1.—Apparatus used for the electrodialysis of soils and soil colloids

This form of cell was found to be very satisfactory for the electrodialysis of soils. The large area of the electrodes and the thinness of the central soil chamber reduced the resistance so that when a

[4] Outlet O_2 is on the side opposite to O_1 but is not shown in the figure.

220-volt direct current was applied to the cell with a 50 to 25 watt lamp in series the temperature did not rise above 50° C. With a central chamber of the dimensions given in Figure 1, approximately 100 gm. of soil or 60 gm. of the colloid could be electrodialyzed at one time.

The parchment paper always contains impurities, especially sulphates. These were removed by filling all the chambers with water and allowing the current to flow for some time with a few changes of water.

The removal of anions is somewhat retarded by the electronegative charge of the soil and parchment membrane. The water at the interfaces being correspondingly electropositive, there is an electroosmotic movement of the liquid toward the cathode which impedes the migration of the anions. The use of an electropositive chromegelatin membrane on the anode side has been recommended by Freundlich (7) to nullify this one-sided electrosmose. There does not seem to be any necessity for using such a membrane when soil materials, which contain a considerable excess of diffusible cations over anions, are subjected to prolonged treatment. It was found that the same proportions of bases and acids were removed from a soil whether the anode membrane was positive or negative. Furthermore, on electrodialyzing a dilute solution of K_2SO_4 it was found that the retardation of the anions is only temporary and does not prevent a quantitative separation. The alkalinity of the cathode solution was considerably higher than the acidity of the anode solution at the end of the first half hour; but after another half hour, when the electrodialysis was completed, a quantitative relationship obtained.

PRELIMINARY EXPERIMENTS ON ELECTRODIALYSIS

Before making quantitative determinations on the colloidal soil material, preliminary tests were conducted with two untreated soils in order to gain an idea of the approximate time required for the electrodialysis to reach an end point. The two soils tested were the Sharkey clay, which is high in monovalent and divalent bases, and the Manor loam, which is comparatively poor in these bases.

The rate at which material was removed was determined by withdrawing the electrodialysates from the cathode and anode chambers hourly and titrating the bases and acids with phenolphthalein as an indicator. A 78.35 gm. sample of the Sharkey soil and a 98.85 gm. sample of the Manor soil were electrodialyzed. The specific conductivity in reciprocal ohms of the Sharkey soil in the pasty condition at 25° C. was 1287×10^{-6} before and 286×10^{-6} after electrodialysis, while the conductivity of the Manor soil under the same conditions was only 152×10^{-6} before and 24×10^{-6} after the treatment.

Table 2 shows the milliequivalents of titrable bases and acid removed hourly from the two soils.

TABLE 2.—*Bases and acids removed hourly by electrodialysis of two soils*

Time of electrodialysis	Sharkey clay soil			Manor loam soil		
	Base	Acid	Appearance of cathode dialysate	Base	Acid	Appearance of cathode dialysate
Hours	*Milliequivalents*	*Milliequivalents*		*Milliequivalents*	*Milliequivalents*	
1	2.655	0.860	Clear	1.810	0.152	Clear.
2	2.340	.255	do	1.180	.062	Light-colored sediment.
3	2.282	.205	do	.410	.050	
4	2.260	.175	do	.110	.042	Do.
5	*2.710	*.320	do	.043	.040	Reddish sediment.
6	2.060	.090	do			
7	2.015	.090	do			
8	1.995	.090	do			
9	1.900	.085	do			
10	1.810	.085	do			
11	1.615	.085	do			
12	*1.830	*.250	Light-colored sediment.			
13	.815	.075	do			
14	.550	.070	do			
15	.420	.065	do			
16	.360	.070	do			
17	.300	.065	Reddish sediment			
18	.260	.070	do			
19	*.410	*.190	do			
20	.140	.060	do			
21	.120	.060	do			
22	.090	.060	do			
Total	28.937	3.375		3.553	.346	

* These relatively high values are the result of standing overnight with no current passing.

It will be noticed that the quantity of titrable, or soluble, base removed hourly from the Sharkey clay soil remains high during the first 12 hours and then falls sharply, but is still determinable at the twenty-second hour. In the case of the Manor loam, a sharp decrease is evident at the end of the second hour. Coincident with the rapid falling off in the quantity of soluble bases, the cathode solution becomes turbid. This turbidity is at first light colored and consists chiefly of $Mg(OH)_2$ and $Al(OH)_3$. Later it becomes brownish due to $Fe(OH)_3$. The appearance of the insoluble bases is evidently governed by the hydrogen-ion concentration in the central chamber containing the soil. The electrodialysis of the Sharkey clay was in one case interrupted at the point where a sediment began to appear, and the P_H, as far as could be determined colorimetrically in the rather turbid soil extract, was found to be 5.4. Owing to the alkaline reaction in the cathode chamber, the cations of insoluble bases must be precipitated as soon as they reach this chamber.

In this experiment no definite end point was apparent in the yield of electrodialyzable material. Subsequent tests showed, however, that when the electrodialysis of the Sharkey soil was prolonged to 42 hours, titrable bases in the hourly extract were less than 0.01 milliequivalent and the yield of insoluble bases had practically vanished. This is a fairly definite end point, although it is approached asymptotically. The soil colloids in the following experiments were therefore electrodialyzed until the hourly cathode extract required less than one drop of N/10 acid for neutralization and contained little or no sediment.

It will be noted that the yield of acids in this experiment was only one-eighth to one-ninth that of the soluble bases. The titrable acid consisted chiefly of H_2SO_4 and H_3PO_4.

While Table 2 shows the rate at which the combined soluble bases are removed from the whole soil, it does not show the rates at which the individual bases are removed from the colloidal material alone. In order to gain information on this point, a sample of the Sharkey colloidal material was electrodialyzed and three successive fractions of the cathode dialysate were analyzed separately.

The Sharkey colloidal material electrodialyzed was not part of the sample, the analysis of which is given in Table 1, but was isolated from the same lot of soil and was very similar in composition. A sample of 54.6 gm. was electrodialyzed for 42 hours, the cathode solution being removed hourly. The last cathode solution required only 1 drop of N/10 acid for neutralization and was about free from sediment. The cathode solutions removed each hour were combined to form three separate fractions that corresponded to the changes observed in the preliminary experiment. The first fraction, A, amounting to 1,850 c. c., contained the first 12 hourly solutions, all of which were clear. Fraction B, of 930 c. c., included the solutions obtained during the 6 succeeding hours and represented the solutions which contained the light-colored sediment. Fraction C, of 3,060 c. c., collected in the last 24 hours, contained all solutions showing an appreciable quantity of brownish sediment.

Table 3 shows the bases present in each fraction, expressed as percentage of the weight of oven-dry colloid.

TABLE 3.—*Successive fractions of bases removed by electrodialysis of the Sharkey soil colloid*

Fraction	Fe_2O_3	Al_2O_3	TiO_2	MnO	CaO	MgO	K_2O	Na_2O
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
A	Trace.	Trace.	None.	Trace.	1.075	0.007	0.091	0.030
B	0.008	0.054	None.	0.003	.137	.136	.017	.009
C	.091	.109	None.	.016	.058	.245	.022	.007
Total	.099	.163	None.	.019	1.270	.388	.130	.046

The first or clear fraction contained most of the lime, potash, and soda removed during the whole period of electrodialysis; only a small quantity of magnesia and traces of the other bases were present. In the second fraction magnesia increased markedly, and alumina, iron, and manganese began to appear in determinable quantities. The last fraction differed from the second in a further increase in the magnesia, iron, alumina, and manganese. The order in which the different bases appear in the cathode chamber is about as follows: Ca, K, and Na; Mg; Al, Mn, and Fe.

This order may not represent the order in which the cations are actually released from the soil colloid particles. The maintenance of Fe, Al, Mn, and Mg in solution and consequently their transport by the current is dependent on the P_H. Under the conditions of the experiment, the P_H in the central chamber is modified by the transport of H and OH ions resulting from the electrolysis of acids and bases in the anode and cathode chambers, respectively. At the beginning

of the experiment the layer in the central chamber adjacent to the cathode parchment is alkaline and the layer adjacent to the anode parchment is acid. Later, as the bases are removed, the reaction becomes acid, throughout the chamber.

PART OF THE BASES IN THE COLLOID REMOVED BY ELECTRODIALYSIS

In order to determine what part of the bases in the soil colloid are removable by the action of the current, samples of the Sharkey and Norfolk soil colloids which had previously been analyzed (Table 1) were electrodialyzed until the hourly cathode dialysate was nearly clear and neutral.

Table 4 shows the quantity of each base removed by electrodialysis, as percentage of the sample; the quantity of each base present, as percentage of the sample; and the proportion of each base removed, as percentage of the quantity present.

TABLE 4.—*Proportion of each base removed by electrodialysis* [a]

Kind of colloid	Determinations	Al_2O_3	Fe_2O_3	MnO	CaO	MgO	K_2O	Na_2O	NH_2
		Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Sharkey	Base removed (as percentage of sample).	0.29	0.14	0.017	1.33	0.37	0.16	0.04	0.12
	Total base present (as percentage of sample).	22.52	8.12	.035	1.36	2.52	1.89	.21	.....
	Proportion of base removed (as percentage of total base present).	1.29	1.72	48.571	97.79	14.68	8.47	19.05	.....
Norfolk	Base removed (as percentage of sample).	.20	.03	.002	.16	.05	.06	.01	.18
	Total base present (as percentage of sample).	33.51	11.24	.005	.30	.44	.49	.25	.....
	Proportion of base removed (as percentage of total base present).	.60	.27	40.000	53.33	11.36	12.24	4.00	.....

[a] Most of the ammonia present was adsorbed by the colloid in the course of its separation from the soil.

It is apparent that the total quantity of bases removable by electrodialysis may vary greatly with different colloids. In the case of the Sharkey colloid the sum of the bases removed amounts to 2.47 per cent of the sample, while in the case of the Norfolk colloid the yield of bases is only 0.69 per cent, or less than one-third as much.

The percentage of each base present which is removed varies less widely for the two colloids than the total quantity removed. With the possible exception of potash, the proportion of base removed to quantity present is greater in the case of the Sharkey colloid than in the case of the Norfolk. This is true even for iron and aluminum, although the Norfolk contains considerably more of these bases. In general, however, the proportion of calcium, magnesium, or manganese removed by electrodialysis is not very different for the two colloids, considering the widely different quantities of these constituents in the two materials.

While the proportion of each base removed is not exactly the same for the two colloids, it is evident that the various bases are characterized by different degrees of removability. The proportion of the lime or manganese and the proportion of alumina and iron

removable is still less. These differences in the comparative removability of the bases probably hold for the colloids of most humid soils, since the Sharkey and Norfolk colloids are representative of widely different groups. Colloids from alkali or salt soils, may, however, show a greater proportion of the sodium replaceable.

MATERIAL REMOVED BY ELECTRODIALYSIS COMPARED WITH THAT REMOVED BY BASE EXCHANGE

It is quite apparent from the work of other investigators on base exchange that various salt solutions extract a fairly definite quantity of bases from the colloidal soil material. A comparison of the bases removed by electrodialysis with those removed by methods in use on base exchange was desirable, since both procedures yield a fairly definite quantity of bases.

As the reagents most commonly used at the present time for determining the exchangeable bases in soils are normal NH_4Cl and 0.05 normal HCl, the exchangeable bases in the Sharkey and Norfolk colloid were determined by these solutions, following the standard procedure described on page 554. The colloidal materials used were portions of the lots that were prepared for the work on electrodialysis.

Table 5 shows the quantities of bases and acids removed from the two colloids by the NH_4Cl and HCl solutions compared with the quantities removed by electrodialysis. The quantities are expressed as percentages of the weight of oven-dried colloid.

TABLE 5.—*Bases and acids extracted from colloidal soil materials by base exchange reagents and by electrodialysis*

Kind of colloid	Treatment of colloid	SiO_2	Fe_2O_3	Al_2O_3	MnO	CaO	MgO	K_2O	Na_2O	P_2O_5	SO_3
		Per ct.	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*
Sharkey	Extraction with N/1 NH_4Cl.	None.	Trace.	Trace.	0.016	1.33	0.36	0.13	0.03	Trace.	(a)
	Extraction with N/20 HCl.	0.14	0.35	0.80	.019	1.39	.41	.14	.03	0.06	0.04
	Electrodialysis	.05	.14	.29	.017	1.33	.37	.16	.04	.04	.04
Norfolk	Extraction with N/1 NH_4Cl.	None.	Trace.	Trace.	.002	.15	.04	.07	.02	Trace.	(a)
	Extraction with N/20 HCl.	0.09	0.05	0.52	.002	.16	.03	.08	.01	0.01	0.04
	Electrodialysis	.02	.03	.20	.002	.16	.05	.06	.01	.01	.04

[a] Not determined.

It is apparent that base exchange methods and electrodialysis remove approximately the same quantities of constituents from the soil colloids. The agreement between the methods is very close, except in the case of alumina, iron, and silica. The greater quantities of these constituents removed by HCl and by electrodialysis than by NH_4Cl is probably conditioned by the P_H of the solution in contact with the colloid. The P_H of the total NH_4Cl extract of the Sharkey colloid was 6.2 and that of the Norfolk 6.1; the P_H of the electrodialyzed colloids was about 5.0; while the P_H of the final HCl extract may be assumed to be the same as that of the N/20 HCl solution, about 1.5. Doubtless the NH_4Cl treatment would have removed some Al and Fe from the Norfolk colloid in its natural condition. The Norfolk colloid is naturally acid; but in isolating

this colloid from the soil a small amount of ammonia was used to promote dispersion and this was apparently sufficient to render the colloid nearly neutral.

In view of the practical identity of the material removed by the different procedures, the conclusions regarding the material removed by electrodialysis also apply to the material removed by base exchange. Briefly, the total quantity of bases removed by base exchange from the two colloids varies widely; the part of each base removed is not the same for the two colloids, but it is sufficiently similar to justify the conclusion that in most colloids lime and manganese are removed to a greater extent than magnesia, potash, or soda, and that a still smaller part of the alumina and iron are removable.

The proportion of one base to another in the materials extracted by electrodialysis and by base exchange is also of interest. This is shown in Table 6 for the monovalent and divalent bases. Iron and aluminum were omitted in this calculation, since such different quantities were extracted by the various methods that the inclusion of these bases would have obscured the relationship that obtains between the other bases. Ammonia was determined only in the electrodialysates and is therefore also omitted.

Table 6 gives the total milliequivalents of Ca, Mg, K, Na, and Mn extracted from 1 gm. of colloid by electrodialysis and by base exchange reagents and the proportion of each base in the total. The bases exchanged with methylene blue, which are included in this table, were determined in connection with another investigation and do not represent the maximum quantity that can be displaced by this reagent, since only a small quantity of the dye was used.

TABLE 6.—*Proportions between the bases in material removed from the colloids by electrodialysis and by base exchange*

Kind of colloid	Treatment	Mn, Ca, Mg, K, and Na extracted per gm. colloid	In 100 milliequivalents were found—				
			Mn	Ca	Mg	K	Na
		M. eq.	*M. eq.*	*M. eq.*	*M. eq.*	*M. eq.*	*M. eq.*
Sharkey	Electrodialysis	0.712	0.67	66.7	26.0	4.8	1.8
	Displaced by N/1 NH_4Cl	.698	.66	68.1	25.8	4.1	1.4
	Displaced by n. 0.05 HCl	.746	.72	66.5	27.2	4.2	1.3
	Displaced by methylene blue	[a] .486	[b] N. D.	65.5	27.6	4.9	2.0
Norfolk	Electrodialysis	.099	0.61	57.6	25.2	13.1	3.3
	Displaced by N/1 NH_4Cl	.096	.62	56.3	30.9	15.6	6.3
	Displaced by n. 0.05 HCl	.093	.65	61.2	16.1	18.3	3.3
	Displaced by methylene blue	[a] .061	[b] N. D.	58.3	20.5	16.4	4.9

[a] Mn not included. [b] Not determined.

It is evident that the proportions between the various monovalent and divalent bases are practically identical in the material removed by the four different methods of extraction within the limits of experimental error.

The proportions between the bases in the Sharkey extract are somewhat different from the proportions in the Norfolk extract. It will be noted, however, that the two colloids differ less widely in the proportions between the bases extracted than in the total quantity

removed or than in the proportions between removable and non-removable bases shown in Table 4.

The proportions between the exchangeable bases in the two colloids are about the same as those reported by other investigators. Apparently these proportions hold approximately for such soils. This is perhaps not surprising, since the river waters of humid regions, such as the Atlantic slopes of North America and western and central Europe, contain about the same equivalent proportions of the monovalent and divalent cations (*5, p. 75, 97*).

DISPLACEMENT OF HYDROGEN FROM ELECTRODIALYZED COLLOIDS

It has repeatedly been shown that when the exchangeable bases in a soil are displaced by the cations of a salt, an equivalent quantity of the displacing ions are retained by the soil. Since the material removed by electrodialysis was identical with that removed by the neutral salt, except for iron and aluminum, it seemed possible that in electrodialysis there might also be an exchange of cations. Furthermore, the fact that in cataphoresis determinations the electrodialyzed colloid behaved in a manner similar to that of the original colloid, showed that it must contain some adsorbed cations. The only cations available to take the place of the cations removed by electrodialysis would be the hydrogen ions of the water.

If hydrogen were adsorbed by the colloid on electrodialysis, it should appear as acid when the material is treated with a neutral salt solution. The Sharkey colloid was therefore leached with 2 liters of a hot four normal KCl solution and the filtrate titrated with tenth normal NaOH, brom thymol blue being used as an indicator. Five grams of the colloid yielded 3.28 milliequivalents of acid to the first liter and only 0.03 milliequivalent to each of the third and fourth half liters. A normal $CaCl_2$ solution gave almost identical results. It is thus evident that neutral salts displace a fairly definite quantity of acidity from the electrodialyzed colloid. The quantities of acid displaced from the Sharkey and Norfolk colloids by 1 liter of normal $CaCl_2$ are given in Table 7, calculated as milliequivalent of hydrogen ion per gram of colloid.

After treatment with the $CaCl_2$ solution, the two colloids were washed free from chlorides and again electrodialyzed to determine how much Ca had been adsorbed in displacing the hydrogen from the electrodialyzed material. The quantities of Ca removed by the second electrodialysis are also given in Table 7.

It will be seen that in the case of the Sharkey colloid the Ca removed by the second electrodialysis was almost exactly equivalent to the acidity produced by the $CaCl_2$ treatment, while in the case of the Norfolk colloid, the Ca removed was slightly greater than the acidity. Evidently the electrodialyzed colloid gives the same type of exchange reaction with neutral salts as the untreated soil or colloid, the only difference being that the electrodialyzed material exchanges hydrogen for the cation of the salt instead of a mixture of monovalent and divalent bases or a mixture of these bases and hydrogen. In the case of the electrodialyzed Sharkey colloid, the exchange capacity is shown by either the acidity liberated or by the Ca adsorbed; but in the case of the Norfolk colloid the Ca adsorbed is probably a more

accurate measure of the exchange capacity than the acidity developed. Work in progress indicates that the discrepancy of 0.036 milliequivalent between acidity developed by $CaCl_2$ treatment and Ca adsorbed is probably due to an adsorption of Cl ions by the electrodialyzed Norfolk colloid.

In Table 7 the exchange capacity of the electrodialyzed colloids is compared with that of the untreated colloid as measured by the monovalent and divalent cations removed in electrodialysis and as determined by the Ca adsorbed from neutral $CaCl_2$.[5]

TABLE 7.—*Exchange capacities of the Sharkey and Norfolk soil colloids before and after electrodialysis*

Determination	Milliequivalent per gram of colloid	
	Sharkey	Norfolk
Ca adsorbed from neutral $CaCl_2$ by the original colloids	0.796	0.207
Total monovalent and divalent cations removed by electrodialysis	.785	.208
Hydrogen displaced from electrodialyzed colloids by $CaCl_2$ solution	.640	.164
Ca adsorbed by electrodialyzed colloids from the $CaCl_2$ solution, determined by a second electrodialysis	.647	.201

It will be seen that the exchange capacities of both colloids are almost the same when measured by Ca adsorbed as when measured by monovalent and divalent bases removed by electrodialysis. The exchange capacity of the electrodialyzed Norfolk colloid is almost identical with that of the untreated material. The exchange capacity of the electrodialyzed Sharkey, however, is about 20 per cent less than that of the original colloid. The results on the whole tend to substantiate the idea that in the process of electrodialysis there is an exchange of hydrogen for all, or most, of the bases removed and the adsorbed hydrogen can in turn be replaced by other cations.

In the preceding considerations of the exchange capacity of the electrodialyzed colloids, the Fe and Al were not regarded as constituting part of the exchangeable cations, although the $CaCl_2$ treatment yielded quantities of the trivalent cations in addition to those already removed by electrodialysis. The electrodialyzed Sharkey yielded 0.458 milliequivalent of combined Al and Fe, 0.353 milliequivalent of which was Al, and the Norfolk colloid gave 0.353 milliequivalent of the trivalent cations, 0.124 milliequivalent being Al. If Fe and Al did exchange with the Ca of the $CaCl_2$ solution, they would of course be hydrolyzed and produce acidity; so both acid and trivalent bases should not be included in calculating the exchange capacity. Inasmuch as the combined Fe and Al in the $CaCl_2$ filtrate was sufficient to account for only approximately two-thirds of the acidity, the acid was apparently the fairer measure of the exchange capacity, even if part (or all of it) resulted from exchanged Fe and Al.

On the whole, it appears as though electrodialysis may be looked upon as a form of base exchange, in which hydrogen ions are substituted for monovalent and divalent bases. While by the usual methods of base exchange in the soil we continually introduce a high

[5] The Ca adsorbed after prolonged leaching of the colloid with neutral $CaCl_2$ solution was determined by subsequent displacement with NH_4Cl.

concentration of the displacing cation and continually withdraw the displaced ions, we arrive in electrodialysis at the same result by the mere withdrawal of the cations, in which case the displacing ion must be the ever-present hydrogen ion of the water. Both methods are in principle the same: A displacement or equilibria either by increasing one factor or by decreasing the other; in one case we apply a push, in the other a pull.

CONDITION OF THE BASES REMOVED BY ELECTRODIALYSIS OR BASE EXCHANGE

It was shown (Table 4) that electrodialysis removed about half the manganese present in the two colloids, most of the calcium, and only a small part of the magnesium, potassium, and sodium. A neutral salt removed almost identical proportions of these bases (Table 5). It is thus apparent that part of each of the monovalent and divalent bases is more reactive than the remainder. This would indicate that each of the monovalent and divalent bases in the colloid is present in two conditions. The condition of the nonreactive part of the bases might be described simply by the facts as nonexchangeable, in the absence of definite information regarding the ultimate chemical constitution of the insoluble part of the soil colloid particle.

The exchangeable bases are usually regarded as being present in the adsorbed condition. From the rapidity of base exchange reaction, Hissink (*13*) concludes that the exchangeable bases are situated on the surface of the soil particles in the adsorbed condition and ascribes the charge of the particles to a partial dissociation resulting in a Helmholtz double layer. Wiegner (*21*) identifies the exchangeable cations with the cations of the outer Helmholtz layer. In fact he calculates what size the primary particles in a clay having an exchange capacity of 3.5 milliequivalents per gram would have to be in order to accommodate all the exchangeable cations in the outer layer.

Wiegner arrives at a value of 7.29 $\mu\mu$ for the diameter of the primary particles which would afford the required surface. However, an error occurs in his calculations in that he assumes the surface per gram of clay varies inversely as the square of the radius instead of inversely as the radius.[6] If this error is corrected, the diameter of the hypothetical primary particles becomes 0.106 $\mu\mu$, or about that of a hydrogen ion. This is obviously absurd.

In a previous publication it was shown that there is an intimate relation between the exchangeable bases and the electrokinetic potential of soil colloids (*18*). Although the exchangeable cations influenced the charge of the particles, it appeared quite improbable that all the exchangeable cations were present in the outer Helmholtz layer. The fact that on neutralizing the charge of the particles with methylene blue the cataphoretic movement was not markedly reduced until most of the exchangeable cations had been replaced indicated that only a part of the cations were dissociated.

From the formula connecting the charge on a particle with the electrokinetic potential, applied by Von Hevesy (*12*), Freundlich (*6, p. 538*), and others to colloidal particles in general and by Wiegner

[6] The surface presented by 1 gm. of colloid is equal to the surface of one particle, $4\pi r^2$, multiplied by the number of particles per gram $\frac{1}{\frac{4}{3}\pi r^3 \times \text{sp. gr.}}$: hence surface $= \frac{3}{r \times \text{sp. gr.}}$

to clay particles, the number of electronic charges on a particle of the Sharkey colloid has been calculated, using the values for the electrokinetic potential and the radius of the particles reported in previous publications (*2*) and assuming a thickness of the double layer of 5 $\mu\mu$.[7] The number of charges in terms of univalent ions or electrons on a particle of the Sharkey colloid was thus found to be 857 ions.

The exchange capacity of the Sharkey colloid at the point of neutrality is about 0.8 of a milliequivalent. This is equal to $4.8 \times 10^{+20}$ ions. Since 1 gm. of the colloid would contain $9.6 \times 10^{+14}$ particles with a radius of 45.5 $\mu\mu$, the number of exchangeable cations per particle is 505,208, or about 590 times the number of cations that should, according to the above calculation, be present in the outer, electropositive layer surrounding the particle. If, therefore, the assumed thickness of the double layer and the size of the particle determined by ultramicroscopic count represent the true values, it is evident that only a very small fraction of the exchangeable bases exist in the dissociated condition.

A thickness of the double layer of 5 $\mu\mu$ is probably too low rather than too high, since the cataphoretic movement was measured in distilled water, under which conditions Gouy (*11*) assumes a much greater thickness. But even if a thickness of the double layer of molecular dimensions were assumed, the charge on the particles would not nearly account for all the exchangeable bases.

It is of course probable that the ultramicroscopic particles of the colloid consist of aggregates of smaller, so-called primary particles. However, the primary particles would have to be 0.077 $\mu\mu$ in radius to afford sufficient surface for 0.8 milliequivalent of ions, if the number of ions per unit surface is assumed to be the same as was calculated for the particle with 45 $\mu\mu$ radius. This value is absurd. Hence, if the theoretical deductions involved in the formulas connecting electrical migration with electrokinetic potential and charge of the particles are correct, it would appear that only a part of the exchangeable bases are present in the dissociated condition.

SUMMARY

Two soil colloids, which are representative of widely different groups of colloidal soil materials, were electrodialyzed in order to determine what part of the bases can be removed by this process. The quantities of bases removed by electrodialysis were compared with the quantities removed by methods commonly used for determining the exchangeable bases in soils.

Preliminary work showed that the quantity of bases that can be removed by electrodialysis is fairly definite and that the order in which the different bases appear in the cathode chamber is about as follows: Ca, K, and Na; Mg; Al, Mn, and Fe. It is pointed out, however, that this may not represent the order in which the cations

[7] The charge, e, on a colloidal particle $= \frac{\zeta D r (r + \delta)}{\delta}$, where ζ is the electrokinetic potential, D the dielectric constant of the dispersion medium, r the radius of the particle and δ the thickness of the double layer. In the case of the Sharkey colloid, $\zeta = 33$ millivolts or $\frac{.033}{300} = 1.1 \times 10^{-4}$ electrostatic unit, and $r = 4.55 \times 10^{-4}$ cm. D is taken as $= 81$ for water and δ is assumed to be 5 $\mu\mu$, or 5×10^{-7} cm., the value generally assumed. The charge for the Sharkey particle becomes 4.09×10^{-7} electrostatic unit. Since one ion has 4.77×10^{-10} electrostatic unit, the charge on the Sharkey particle would be that of 857 ions.

are released from the colloid particles, since the appearance of the bases in the cathode chamber is largely affected by the hydrogen-ion concentration in the compartment containing the colloid.

The total quantity of bases that electrodialysis removed from one colloid was about five times that removed from the other. While the percentages of the Ca or Na in the colloid that are removable do not agree closely in the case of the two colloids, it is evident that the various bases are characterized by different degrees of removability. The proportion of the total lime or manganese removable by electrodialysis is much greater than the proportion of magnesium, potassium, or sodium, and the proportion of the total aluminum or iron removable is still less.

Extraction of the two colloids with normal NH_4Cl or $\frac{N}{20}HCl$ yields quantities of the monovalent and divalent bases that are almost identical with those obtained by electrodialysis.

Treatment of the electrodialyzed colloids with a $CaCl_2$ solution develops quantities of acidity that approximate the base exchange capacities of the untreated colloids. It appears that in the process of electrodialysis there is a substitution of hydrogen ions from the water for most of the monovalent and divalent cations removed by the electric current.

It is evident that each of the monovalent and divalent bases in the colloid is present in two conditions which might be defined simply as exchangeable and nonexchangeable.

The quantity of the exchangeable bases that can exist as cations in an outer Helmholtz layer surrounding the particle is considered. It is pointed out that if the deductions involved in formulas connecting electrical migration with electrokinetic potential and charge of the particles are correct, only a part of the exchangeable bases in the colloid are present in the dissociated condition.

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A CHEMICAL AND PHYSIOLOGICAL STUDY OF MATURITY IN POTATOES[1]

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INTRODUCTION

The problem of maturity in potatoes assumes considerable practical importance in connection with several claims for the superiority of immature potatoes for seed. The term "immature seed" expresses a rather vague idea on account of our lack of a definite and scientific conception of what constitutes maturity in potatoes.

One object of the experiments reported in this paper was to determine the character of the chemical changes in potatoes while they are ripening and maturing on the vines and to correlate, if possible, the different stages of maturity with certain characters of the vines. The chief information sought was the extent to which the chief maturing processes in the tubers may continue in storage. Will potatoes apparently immature but large enough for seed attain during storage the percentage composition of solids and the physiological condition characteristic of tubers allowed to fully mature on the vine? The nitrogenous constituents are specially emphasized in this paper.

EXPERIMENTAL PROCEDURE

The seed used for the experimental crop was Irish Cobbler grown in Garrett County, Md. At intervals, beginning when the vines were in bloom, four to six typical hills were dug and the tubers divided into two lots matched for size and number of tubers. One lot was sampled immediately for analysis and the other was placed in cellar storage at a fairly constant temperature of 68° F. The storage lots were all sampled for analysis at the same time; that is, at the end of the rest period on October 26.

The dates on which the tubers were dug and the condition of the plants on each of these dates were as follows:

June 17, plants in full blossom.
June 24, blossoms gone, but leaves green.
July 8, tips of leaves beginning to die; vines prone.
July 15, about 80 per cent of the leaves dead.
July 22, leaves all dead; vines partly dead.
August 27, vines brown and dry.

The average number and weight of the tubers per hill and the percentage of loss in weight of the different lots during the storage period are given in Table 1.

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[2] The writers acknowledge the able assistance of C. M. Conrad, of this station, who verified some of the analytical methods and completed some of the later analyses.

TABLE 1.—*Average number and weight of tubers per hill on the different digging dates, and loss in weight of the different lots during the storage period*

Date of digging	Average number of different sized tubers per hill					Total number	Total weight per hill in ounces	Percentage loss in weight on Oct. 26
	Pea	Marble	Walnut	Egg	Larger			
June 17	9.3	2.3	4.2	1.9	0	17.6	5.5	16.45
June 24	10.2	3.0	2.6	3.0	0	18.8	7.3	12.38
July 8	8.6	4.3	2.0	3.3	2.6	21.0	20.6	8.67
July 15	8.0	1.5	3.5	1.5	8.0	22.5	26.1	6.70
July 22	2.5	1.6	3.0	3.0	5.6	15.6	28.0	6.10
Aug. 27	2.0	1.6	4.6	3.3	4.3	16.0	26.8	4.62

ANALYTICAL METHODS

SAMPLING AND DETERMINATION OF MOISTURE

The tubers were ground to a pulp in a meat grinder, and after thorough mixing samples of the fresh pulp were weighed out for moisture, sugars, and nitrogenous constituents.

To determine the moisture, about 5 gm. of pulp were dried to constant weight at 70° C. in a vacuum of 30 inches of mercury.

SUGARS

For the determination of sugars a sample of 50 gm. was weighed into a counter-poised Kohlrausch sugar flask into which had been placed 0.25 gm. of $CaCl_2$. The pulp was covered immediately with 75 c. c. of 95 per cent boiling alcohol and the mixture brought to boiling on the steam bath, after which sufficient hot water was added to bring the extraction medium to 50 per cent alcohol by volume. After boiling five minutes the samples were allowed to stand overnight in the 50 per cent alcohol and the volume then completed to the mark with 95 per cent alcohol and stored. After the alcohol had been removed the sugars were determined in the aqueous solution acording to the Munson and Walker method.

ASH, STARCH, AND CRUDE FIBER, TOTAL NITROGEN

The ash was determined by igniting the residues from the moisture determinations. The percentage of ash in the original moist sample was calculated by indirect proportion.

The starch and crude fiber were determined by the official methods of the Association of Agricultural Chemists, using oven-dried material. The diastase method with subsequent acid hydrolysis was employed for starch.

From 8 to 9 gm. of fresh pulp were weighed from a weighing bottle with ground-in glass stopper and the total nitrogen determined by the official Gunning method.

DISTRIBUTION OF NONPROTEIN NITROGEN

The possibility of autolytic changes is always present during aqueous extractions of the soluble nonprotein nitrogenous constituents in fresh plant tissues. This objection could be eliminated by using alcohol of sufficient strength to prevent autolytic changes if complete extraction of these constituents is possible in such a medium. Bacterial action and filtering difficulties would also be largely avoided.

Jodidi [3] extracted the amino acids of rye flour directly with boiling 92 per cent alcohol. He found an average of 20.43 per cent monoamino nitrogen in one variety and 35.07 per cent in another variety when calculated on the basis of alcohol-soluble nitrogen. These percentages were higher than those found in the aqueous extracts of the same varieties, based upon water-soluble nitrogen. However, he does not state the relative amounts of either total soluble nitrogen or total nonprotein nitrogen obtained by the water and alcohol extractions in this experiment. In a previous experiment he found that water extracted about three times as much nitrogen as did the alcohol. He states that this is perhaps due to the greater proportion of protein matter taken up by the water. The weight of the sample used for the alcohol extraction was four times that for the water extraction. Then, too, all of the nonprotein nitrogen may not be soluble in the strong 92 per cent alcohol used for the extractions.

Osborne, Wakeman, and Leavenworth [4] precipitated the protein of alfalfa juice with alcohol of 53 per cent by weight. They assume that the nonprotein nitrogen is soluble in this percentage of alcohol, although they state that their evidence is not entirely convincing to show that a small part of the precipitate may not belong to nonprotein substances insoluble in 53 per cent alcohol. In later work by Vickery [5] the nonprotein nitrogenous constituents of the juice of the alfalfa plant were determined in the filtrate from a 53 per cent alcoholic solution.

Preliminary experiments were conducted to test the effectiveness of alcoholic extractions as compared with aqueous extractions of the soluble nonprotein nitrogenous constitutents in potatoes. The results in Table 2 are based upon the following detailed procedures for both types of extractions:

TABLE 2.—*Percentages of nonprotein nitrogen in potatoes obtained by water and alcoholic extractions*

Sample No.	Water extract	Alcoholic extract
1	0.226	0.217
2	.227	.218

The tubers were cooled and then grated on a nutmeg grater in a mortar surrounded by cracked ice. By this means the pulp was maintained at a temperature of 4° C. during sampling.

For the water extractions, samples of pulp of 100 gm. each were stirred with 100 c. c. of water and strained through a small coffee strainer. By gently pressing the pulp with a spatula, most of the starch and cell colloids, which make filtering of the pulp difficult, were removed. The pulp was transferred with 200 c. c. of water to a smooth filter in a Büchner funnel. Suction was applied after the addition of each portion of water. The filtrate and strained juice

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were combined and sufficient 95 per cent alcohol added to make 53 per cent by weight. The pulp was dried at 70° C. and ground to pass a 40-mesh sieve. The material which weighed about 20 gm. was extracted with 300 c. c. of ammonia-free water by agitating on a mechanical shaker for two hours. Toluol was used as an antiseptic. The material was filtered in a Büchner funnel and thoroughly washed. Alcohol was then added to the filtrate to make 53 per cent by weight. This was combined with the first alcoholic solution. A flocculent precipitate which appeared upon the addition of alcohol was filtered off through paper pulp in a Büchner filter. The alcohol was added to preserve the extracts and also to test the completeness with which this percentage of alcohol would precipitate the soluble protein in a potato extract. Osborne [6] claims that this percentage of alcohol will effect complete precipitation of the soluble protein from an aqueous extract of alfalfa. In these potato extracts the precipitation was far from complete, as shown by the heavy precipitate obtained later by lead acetate.

Similar samples of pulp of 100 gm. each were used for the alcohol extractions. The samples were covered immediately with 400 c. c. of boiling 95 per cent alcohol and stored for one week. The final concentration of the storage alcohol was approximately 75 per cent. The flasks were shaken occasionally during the storage period.

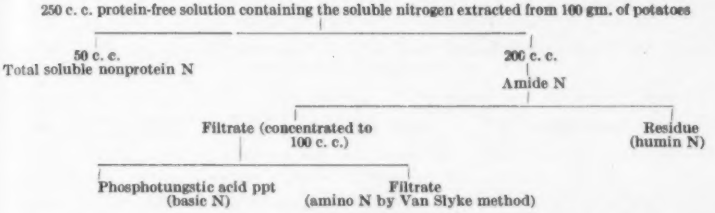
The storage alcohol was filtered through a smooth filter paper in a Büchner funnel and the pulp transferred to the funnel, 50 c. c. of 50 per cent alcohol being used to wash out the flask. After as much of the alcohol was removed as was possible by suction, the pulp was dried in the funnel at 70° C. and ground to pass a 40-mesh sieve. It was then transferred to the original flask and covered with 200 c. c. of hot 50 per cent alcohol by volume. The flask was shaken several times during a period of 24 hours, after which the alcohol was decanted on the same filter and another 200 c. c. of hot alcohol of the same strength added to the flask. After the flask had been shaken frequently for a period of two hours the entire contents were transferred to the filter, and 100 c. c. of 50 per cent alcohol was used to wash out the flask. Suction was applied after the addition of each portion of alcohol. A third extraction with 50 per cent alcohol did not remove any additional nitrogen.

Both the alcoholic and aqueous extracts were concentrated on the steam bath and the soluble protein precipitated with basic lead acetate. The excess of lead was removed by hydrogen sulphide and the final filtrates were made up to 500 c. c. The total nitrogen was determined in 100 c. c. aliquots.

Since the final procedure for alcoholic extraction yielded practically the same amounts of nonprotein nitrogen as the aqueous extractions, it was adopted for the analyses of the research material. The only deviation from this procedure was the period of storage in the 75 per cent alcohol. This period was much longer than that of the preliminary experiments. The total amount of nonprotein nitrogen found in the preliminary experiments was larger than the maximum found in the material under investigation, but the material was not comparable since much older potatoes of a different crop were used

[6] OSBORNE, T. B., WAKEMAN, A. J., and LEAVENWORTH, C. S. THE WATER-SOLUBLE CONSTITUENTS OF THE ALFALFA PLANT. Jour. Biol. Chem. 53: 411–429. 1922.

in the preliminary experiments. The following graphic scheme will serve to indicate the nitrogen fractions determined in the protein-free solutions.


250 c. c. protein-free solution containing the soluble nitrogen extracted from 100 gm. of potatoes
50 c. c.
Total soluble nonprotein N
200 c. c.
Amide N
Filtrate (concentrated to 100 c. c.)
Residue (humin N)
Phosphotungstic acid ppt (basic N)
Filtrate (amino N by Van Slyke method)

The 200 c. c. aliquot of the protein-free solution was acidified with 20 c. c. of concentrated HCl and boiled under a reflux condenser for two hours. The acid was removed by evaporating the solution almost to dryness on a water bath. Calcium hydroxide was used for the distillation of the ammonia from the hydrolyzed acid amides. The clear supernatant liquid above a 10 per cent suspension of calcium hydroxide furnished sufficient alkali without diluting the solution to too great a volume. An excess of calcium hydroxide interfered with subsequent digestions. Duplicate determinations were made in which reduced pressure was used for both the removal of the HCl and the distillation of the ammonia, but this precaution was found to be unnecessary with these solutions.

The basic nitrogenous constituents in the filtrate from the humin nitrogen residue were removed according to the usual procedure with phosphotungstic acid. This reagent also precipitates any proteoses or peptone that may be present.

The phosphotungstic acid precipitate was washed with a solution containing 2.5 per cent phosphotungstic acid and 3.5 per cent HCl and cooled to 0° C. in order to avoid as far as possible the danger of dissolving any of the phosphotungstates of the hexone bases. The phosphotungstic acid precipitate was oxidized by the Kjeldahl method without removing the phosphotungstic acid, as it was found that this did not interfere if the digestion was continued for three hours after the solution is clear.

The combined filtrate and washings from the phosphotungstic precipitate was just neutralized with NaOH, cleared with a little acetic acid and concentrated to 100 c. c. Aliquots of 10 c. c. were used to determine amino nitrogen by the Van Slyke micromethod.

ANALYTICAL RESULTS

The data obtained from all of the analytical work are tabulated in Tables 3 to 9. The distribution of nitrogen is also shown graphically in Figure 1.

TABLE 3.—*Percentage composition of potatoes dug at different stages of development and analyzed immediately*

Date of digging	Moisture	Ash	Crude fiber	Proteins (N x 6.25)	Total sugars	Starch
June 17	83.66	0.91	0.34	1.77	1.03	10.57
June 24	82.72	.91	.30	1.85	1.03	11.47
July 8	80.80	.91	.32	1.82	.68	13.80
July 15	79.35	1.00	.31	2.23	.30	14.98
July 22	80.20	1.02	.31	2.24	.19	14.25
Aug. 27	81.02	1.03	.32	2.17	.26	13.60

TABLE 4.—*Percentage composition of potatoes dug at different stages of development and analyzed at the end of the rest period, October 26*

Date of digging	Moisture	Ash	Crude fiber	Proteins	Total sugars	Starch
June 17	81.26	1.20	0.47	2.00	0.41	10.82
June 24	81.78	1.10	.43	1.88	.41	11.68
July 8	80.09	1.05	.37	1.93	.31	13.34
July 15	78.33	1.05	.37	2.18	.24	14.76
July 22	79.56	1.06	.35	2.33	.19	14.23
Aug. 27	79.65	1.11	.33	2.28	.21	15.05

TABLE 5.—*Percentage composition of Irish Cobbler potatoes dug at different stages of development and analyzed at the end of the rest period*

[Results calculated on basis of moisture content at time of digging]

Date of digging	Ash	Crude fiber	Proteins	Total sugars	Starch
June 17	1.05	0.41	1.75	0.36	9.44
June 24	1.05	.40	1.79	.39	11.08
July 8	1.02	.36	1.86	.30	12.87
July 15	1.00	.35	2.08	.23	14.16
July 22	1.03	.34	2.26	.19	13.78
Aug. 27	1.03	.31	2.13	.20	14.03

TABLE 6.—*Percentage of sugars in Irish Cobbler potatoes at different stages of development and at the end of the rest period*

Date of digging	Percentage of sugars in potatoes—					
	At time of digging			At end of rest period		
	Reducing sugars	Sucrose	Ratio	Reducing sugars	Sucrose	Ratio
June 17	0.203	0.788	3.88	0.273	0.138	0.50
June 24	.146	.904	6.19	.278	.122	.44
July 8	.113	.560	4.96	.209	.103	.49
July 15	.102	.194	1.90	.077	.156	2.02
July 22	.043	.140	3.26	.042	.141	3.35
Aug. 27	.056	.199	3.55	.064	.144	2.25

TABLE 7.—*Percentage of total and nonprotein nitrogen in oven-dried potato pulp*

Date of digging	Percentage of total nitrogen—		Percentage of total nonprotein nitrogen—	
	At time of digging	At end of rest period	At time of digging	At end of rest period
June 17	1.73	1.67	0.400	0.459
June 24	1.72	1.65	.307	.503
July 8	1.52	1.54	.316	.494
July 15	1.73	1.61	.384	.481
July 22	1.81	1.82	.468	.651
Aug. 27	1.83	1.80	.590	.589

TABLE 8.—*Distribution of nonprotein nitrogen in potatoes dug at different stages of development and analyzed immediately*

[Data expressed in percentage of total nitrogen]

Date of digging	Total nonprotein nitrogen	Nitrogen of acid amides	Nitrogen in phosphotungstic precipitate	Monoamino nitrogen
June 17	23.06	3.73	3.60	8.94
June 24	17.84	3.40	3.09	7.34
July 8	20.78	4.07	3.50	8.83
July 15	22.20	4.74	3.87	11.36
July 22	27.07	5.54	4.06	12.00
Aug. 27	32.18	6.58	5.49	15.05

TABLE 9.—*Distribution of nonprotein nitrogen in potatoes dug at different stages of development and analyzed at the end of the rest period*

[Data expressed in percentage of total nitrogen]

Date of digging	Total nonprotein nitrogen	Nitrogen of acid amides	Nitrogen in phosphotungstic precipitate	Monoamino nitrogen
June 17	27.42	4.23	2.10	12.10
June 24	30.39	3.73	2.28	14.83
July 8	32.08	4.69	2.18	15.92
July 15	29.82		2.00	14.57
July 22	35.58	7.24	2.11	18.67
Aug. 27	31.67	5.89	2.55	15.64

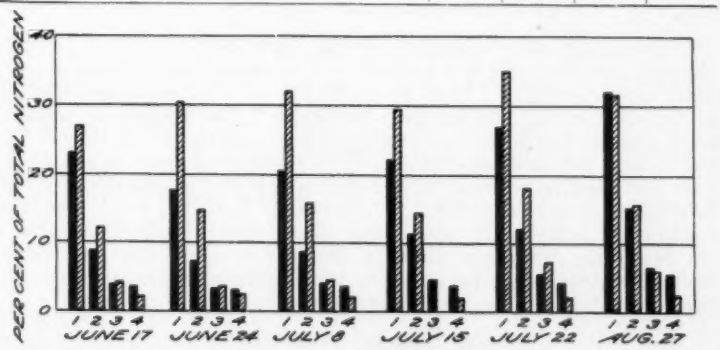


FIG. 1.—Distribution of nonprotein in potatoes at different stages of development (solid black bars) and at the end of the rest period (hatched columns). The first pair of bars of each set represents total nonprotein nitrogen, the second pair monoamino nitrogen, the third pair amide nitrogen, and the fourth pair basic nitrogen. The dates indicate the time of digging

RESPIRATION IN MATURE AND IMMATURE POTATOES

Since respiration is the process which furnishes the energy for growth, it seemed of interest in this connection to compare the respiratory rates in mature and immature potatoes.

Experiment 1: A lot of immature Rural New Yorker potatoes was dug October 3. Another lot was allowed to mature in the ground and was dug October 30. The respiration rate at 22° C. was determined in both lots immediately after digging. The results are shown in Table 10.

TABLE 10.—*Respiration of mature and immature Rural New Yorker potatoes immediately after digging*

Condition of potatoes	Mgm. of CO_2 per kilo per hour (average for succeeding 24-hour periods)										
	First period	Second period	Third period	Fourth period	Fifth period	Sixth period	Seventh period	Eighth period	Ninth period	Tenth period	Eleventh period
Mature	20.4	18.1	20.0	19.4	13.3	11.1	10.7	7.1	6.8	6.3	7.4
Immature	33.1	23.9	20.1	15.4	13.3	20.6	18.0	13.3	11.1	9.8	8.7

Experiment 2: Samples of the same lots of potatoes used in experiment 1 were stored at 36° F. for four months. Respiration rates in the mature and immature potatoes at 22° C. were again compared. The results of this experiment are shown in Table 11.

TABLE 11.—*Respiration of mature and immature Rural New Yorker potatoes at the end of the rest period*

Condition of potatoes	Mgm. of CO_2 per kilo per hour (average for succeeding 24 hour periods)								
	First period	Second period	Third period	Fourth period	Fifth period	Sixth period	Seventh period	Eighth period	Ninth period
Mature	19.3	23.0	22.1	20.7	18.8	15.6	14.1	13.0	10.8
Immature	18.6	22.9	23.0	21.2	18.0	15.5	13.3	11.8	10.8

Other experiments similar to the ones here recorded have all shown greater respiratory activity in the immature potatoes immediately after digging. This is probably due to the greater permeability of the tender skin to gaseous exchange. The immature potatoes were also more subject to skinning and wounding in handling. This would also increase respiration. The respiration rates in the immature and mature potatoes at the end of the rest period are of special interest in this connection. The data in Table 11 show the same respiratory activity in both lots. The higher respiration rates at the beginning are due to the fact that the potatoes were transferred from the storage temperature to the higher temperature at which the respiration determinations were made.

DISCUSSION OF RESULTS

Reducing sugars and sucrose decreased as starch increased up to the time when about 80 per cent of the leaves were dead, but the

tubers were still quite immature as judged by the skin. Sucrose predominated at all stages of development, but the ratio of sucrose to reducing sugars was highest when the tubers were making their most rapid growth. The percentage of sucrose in the immature potatoes decreased during storage so that by the end of the rest period it was practically the same in all lots. The percentages of reducing sugars which are all very low show little change during storage when calculated to basis of moisture content at time of digging.

The small percentage of starch in the very immature lot after storage is probably due to the high respiration of these tubers immediately after digging.

When calculated to a dry basis the percentages of ash, crude fiber, and total nitrogen remain fairly constant at all stages of development. The increase of nonprotein and amino nitrogen in the tubers with increasing periods on the vine show that hydrolysis or digestion of protein is one of the important ripening or maturing processes. These processes in the immature seed continued in storage so that by the end of the rest period the percentages in all lots except the one containing very immature potatoes, were practically the same and equal to those found in the mature tubers immediately after digging.

The higher amounts of nitrogen in the phosphotungstic acid precipitates of the extracts from the tubers at the time of digging may be due to proteoses and peptones which are hydrolyzed to amino acids during storage. The nitrogen of acid amides increases slightly as the tubers mature. The humin nitrogen which is formed during the hydrolysis of the acid amide nitrogen was practically the same in all lots. Since this fraction is not formed in the living tissue and is not significant in this connection the data are not recorded.

The higher respiration in immature potatoes for a period after digging appears to be due to the fact that the skins are more permeable to gases. At the end of the rest period when growth is possible, respiration is no greater in the immature than in the mature seed.

SUMMARY AND CONCLUSIONS

The ripening and maturing processes in potatoes may continue during storage, so that by the end of the rest period immature potatoes large enough for seed have practically the same percentage composition and respiratory response as potatoes allowed to mature on the vine if both are stored under the same conditions.

The data in this paper do not reveal any chemical or physiological basis for the superiority of immature potatoes for seed. The cases reported of immature seed giving better results than mature seed may have been due to greater freedom from degeneration diseases in the immature seed.

THE INFLUENCE OF THE ENVIRONMENTAL TEMPERATURE ON THE HEAT PRODUCTION OF CATTLE [1]

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INTRODUCTION

The study herein reported was suggested by variations in the heat increment per unit of feed, especially as influenced by very low planes of nutrition, which were disclosed by a recent review and recalculation of the published energy metabolism work of this institute (*4*).[2]

A detail of the former experimental procedure of the institute in its respiration calorimetric work with steers, which gave especial significance to the environmental temperature in relation to the heat production of the experimental subject, was the shearing of the steer at the beginning and at the end of the season's program of experimentation to permit of an accounting for the growth of hair. Obviously this procedure would have the effect of raising the critical temperature—that is, the temperature at which the waste heat of food utilization is just sufficient to maintain the body temperature without increasing the katabolism of body substance.

Among the published experiments the one which shows most clearly the relation of environmental temperature to heat production is experiment No. 186, on the available energy of red clover hay. The results of this experiment were first published in 1908 (*1*), and have been discussed from time to time in later papers, with recalculation of the data in the light of progressive improvements of methods and understanding (*2, 4, 6*).

The present paper consists of (1) a brief review of experiment No. 186, the revised results of which have not previously been discussed in detail in relation to critical temperatures, and (2) a report of a later experiment, No. 235, conducted in 1924–25.

EXPERIMENT NO. 186

Experiment No. 186 was conducted during January, February, and March, 1904. The subject was a grade Shorthorn steer, approximately 5 years old, and the feed was red clover hay. The dates included in the experimental periods and the number of days in the calorimeter are given below.

Experimental periods	Days in calorimeter	
	At 19° C.	At 13.5° C.
1, Jan. 13–22	Jan. 13 and 14	Jan. 20 and 21.
2, Feb. 10–19	Feb. 10 and 11	Feb. 17 and 18.
3, Mar. 9–18	Mar. 9 and 10	Mar. 16 and 17.

[1] Received for publication Apr. 22, 1926; issued September, 1926.
[2] Reference is made by number (italic) to "Literature cited," p. 589.

A summary exhibit of the results comprises Table 1.

TABLE 1.—*Experiment 186: Increments of heat production per kilogram of dry matter of feed, as affected by environmental temperature*

Period No.	Temperature of chamber	Dry matter of feed	Metabolizable energy of ration	Heat production, corrected to 12 hours' standing	Gain of energy by animal	Latent heat of water vapor, per cent of total [a]	Heat increments per kgm. of dry matter	
							Periods compared	Calories
	° C.	*Kgm.*	*Cal.*	*Cal.*	*Cal.*			
1a	19.0	2.933	5,922	10,597	−4,675	21.27	1a and 2a	321
1b	13.5	2.933	5,922	11,321	−5,399	14.51	1a and 3a	7
2a	19.0	5.025	10,690	11,268	−578	24.34	1b and 2b	−99
2b	13.5	5.025	10,690	11,113	−423	17.44	1b and 3b	−534
3a	19.0	4.139	8,614	10,605	−1,991	25.35	2a and 3a	748
3b	13.5	4.139	8,614	10,677	−2,063	18.85	2b and 3b	492

[a] Uncorrected to the standard day.

In period 1 as the temperature was decreased from 19° to 13.5°, the quantity of feed remaining the same, the heat production increased from 10,597 to 11,321 Calories, the negative balance of energy increased from 4,675 to 5,399 Calories, and the latent heat of water vapor decreased from 21.27 to 14.51 per cent of the total heat production (uncorrected to the standard day). Obviously the temperature was below the critical in period 1b. That this is true is also shown by the negative heat increments, computed through comparisons involving the heat production of this period.

It is also clear that the temperature was below the critical in period 1a, as is shown by the facts that the heat increments involving period 1a are very much smaller than those derived from periods 2 and 3, and that the heat production is practically the same in period 1a as in period 3a, while the feed is materially less.

That the temperature in periods 2 and 3 was above the critical is clearly shown by the practical identity of the heat production at the two temperatures.

One condition which doubtless affected the critical temperature for this experimental subject, and at a progressively changing rate, was the growth of the hair coat. Since the animal was sheared at the beginning of the experiments (January 2), and the coat was thereafter allowed to grow, the effect of this increasing protection must have been to lower the critical temperature. The results of experiment 235 (to be discussed) give us reason to believe that the difference in the critical temperature for an animal immediately after shearing and two and one-half months afterward, as shown in experiment 186, is a factor of very great importance in determining the influence of environmental temperatures on the production of heat.

A study of the data obtained in experiment 186 makes clear the fact that neither the energy balance nor the percentage of the heat production which is represented by the latent heat of water has by itself a definite diagnostic significance in relation to the critical temperature, though the conditions prevailing in respect to both contribute to the general picture of the response of the animal to the temperature of its environment.

From the point of view of the general program of research on energy metabolism at this institute the important conclusion to be drawn

from this experiment is that even when receiving a considerable quantity of feed (2.933 kgm. of dry matter) an environmental temperature as high as 19° C. may be below the critical temperature for a steer that has been sheared. On account of the imperfect method used for computing the results, especially the heat production as observed to the standard day as to standing and lying, this point was missed in the earlier discussions of this experiment, in spite of the fact that the work was planned especially to bring out the facts in this relation.

The heat increments derived from the three periods at 19° C. were 321 Calories, 7 Calories, and 748 Calories. Except for such error as there may be in the assumption that the heat increment is a lineal function of the feed, theoretically, the only reasons (aside from uncontrolled variations) that these values should not have agreed exactly were that the temperature in periods 1a and 1b was below the critical temperature, and that the steer began the season's experiments without his coat and finished with a two and one-half months' growth of hair.

In other of the early experiments with steers at this institute there were many instances of a loss from the body of as much as 2,000 Calories a day, and in all cases the steers began the season's experimental program without their coats. It is impossible to estimate the influence which the growth of hair during the course of the experiments may have exerted on the results, but that the results were influenced by this added protection to the body of the animal must be considered as at least a possibility, and may be guarded against in the future by not shearing the experimental subjects. It has been found that the error thus introduced into the computed heat production by the failure to account for the growth of hair is properly negligible in studies of the net energy value of feeds.

EXPERIMENT NO. 235

OBJECT

The purpose of experiment 235 was to study the following problems, only the first of which is discussed in this paper: (1) The heat production of the beef steer as affected by the coat of hair and the temperature of the environment; (2) the fasting katabolism as a measure of the maintenance requirement of energy; (3) the comparative utilization of energy for maintenance and body increase; (4) a comparison of the direct and the respiratory quotient methods of measurement of heat production (*a*) during fasting, (*b*) while on a maintenance level, and (*c*) in body increase.

EXPERIMENTAL SUBJECTS

Two steers, designated Nos. 259 and 260, were used in the experiments, No. 259 being an Aberdeen Angus-Galloway cross, aged 16½ months, and No. 260, Aberdeen Angus-Shorthorn cross, aged 15¼ months at the beginning of the experiments. Both steers were of typical beef character, their sires and dams being purebred. Both were quiet and admirably adapted to experimental use. In condition they were approximately half fat. No. 259 was kept shorn throughout the experiments, while No. 260 wore his full winter

coat of hair. The steers had been halter broken and familiarized with the calorimeter and all conditions attendant upon the experimental routine before the experiments began. The live weights of the steers are given in Table 2.

TABLE 2.—*Schedule of experiment No. 235*

Treatment	Steer 260 (full winter coat)			Steer 259 (sheared)		
	Experimental periods	Temperature	Initial live weight	Experimental periods	Temperature	Initial live weight
		° *C.*	*Kgm.*		° *C.*	*Kgm.*
I. Fasting, total duration.	Jan. 9 to Jan. 19[a]			Jan. 27 to Feb. 7[b]		
Calorimeter period No. 1.	Jan. 13, 6 p. m. to Jan. 14, 6 p. m.	15.54	356.8	Feb. 1, 6 p. m. to Feb. 2, 6 p. m.	13.68	349.1
Calorimeter period No. 2.	Jan. 15, 6 a. m. to Jan. 16, 6 a. m.	18.46	354.4	Feb. 3, 6 a. m. to Feb. 4, 6 a. m.	15.70	346.1
Calorimeter period No. 3.	Jan. 16, 6 p. m. to Jan. 17, 6 p. m.	21.51	352.1	Feb. 4, 6 p. m. to Feb. 5, 6 p. m.	18.26	343.1
Calorimeter period No. 4.	Jan. 18, 6 a. m. to Jan. 19, 6 a. m.	14.16	349.7	Feb. 6, 6 a. m. to Feb. 7, 6 a. m.	22.06	340.0
II. Maintenance, digestion period.	Feb. 10 to 27, inclusive.			Feb. 24 to Mar. 13, inclusive.		
Calorimeter period.	Feb. 24, 25, and 26	17.73	378.7	Mar. 10, 11, and 12	17.73	357.7
III. Production, digestion period.	Mar. 10 to 27, inclusive.			Mar. 24 to Apr. 10, inclusive.		
Calorimeter period.	Mar. 24, 25, and 26	17.69	411.7	Apr. 7, 8, and 9	17.76	386.1

[a] Physic given at 9 and 11 a. m., on Jan. 9; last feed, grain only, given on evening of Jan. 9; paunch pumped out and enema given at 11 a. m., Jan. 10.
[b] Last feed, grain only, given on evening of Jan. 27; physic given at 9 and 11 a. m., Jan. 28.

SCHEDULE OF EXPERIMENTS

In the schedule of experiments as set forth in Table 2 it will be noted that both steers were subjected to the same routine, which embraced observations on the three planes—fasting, maintenance, and production.

The fasting period was 10½ days for No. 259 and 9½ days for No. 260. After a preliminary fast of 5 days with No. 259 and 4 days with No. 260, a series of four 24-hour calorimeter experiments, separated by 12-hour intervals, each period being at a different temperature, were conducted with each steer.

The maintenance and production periods were each 18 days in length. During the first 15 days of these periods the steers were confined in digestion stalls, and the feed eaten and the excreta voided were quantitatively accounted for. The last 3 days of each 18-day digestion experiment constituted the calorimeter periods.

PREPARATION OF SUBJECTS

At the beginning of the experiments it was desired to get the steers onto a true fasting basis in the shortest possible time. The method followed with No. 260 was to give a physic and to withhold roughage for 1 day and grain for one-half day; the paunch was then washed out by means of a stomach pump and an enema given. The pump showed the paunch to be empty at this stage; hence in the preparation of No. 259 the pumping out of the paunch was omitted.

The preliminary feeding was on a maintenance basis. After the termination of the 9½ and 10½ day fasts the steers ate very little for a few days; in fact, in both cases it required 4½ days to get them back to as much as a maintenance ration.

Steer No. 259 was sheared January 27 and again March 4. The weight of the full winter coat as removed January 27 was 1,200 gm., the steer weighing at that time 373 to 384 kgm. (before and after watering). On March 4, 36 days thereafter, a second shearing yielded 52 gm. of hair. The growth of hair, therefore, seems to have been at the rate of 1.4 gm. per day.

METHODS OF EXPERIMENTATION

The methods of work conformed, in a general way, to those previously used at the institute, as detailed in various publications, and with those outlined by Forbes and Grindley (*3*). However, since the publication in 1918 of the last article on steer feeding such material improvements of procedure have been accomplished in the institute's respiration calorimetric studies as to place them on a new status of accuracy and promise. These changes of procedure have recently been enumerated in "Science" by one of the present writers (*5*).

TABLE 3.—*Experiment 235: Daily heat production of fasting steers*

Animal and temperature of chamber (° C.)	Period	Heat emission			Heat emission corrected to one-half time standing	Heat production corrected to one half time standing
		By radiation and conduction	As latent heat of water vapor	Total		
		Cal.	*Cal.*	*Cal.*	*Cal.*	*Cal.*
Steer No. 260 (full coat):						
15.54	1a	2,411	878	3,289	3,347	----------
	1b	2,122	704	2,826	2,904	----------
	Total per day	4,533	1,582	6,115	6,251	6,322
18.46	2a	2,047	997	3,044	3,126	----------
	2b	2,060	1,190	3,250	3,330	----------
	Total per day	4,107	2,187	6,294	6,456	6,430
21.51	3a	1,839	1,391	3,230	3,315	----------
	3b	1,725	1,400	3,125	3,171	----------
	Total per day	3,564	2791	6,355	6,486	6,453
14.16	4a	2,240	797	3,037	3,117	----------
	4b	2,279	746	3,025	3,114	----------
	Total per day	4,519	1,543	6,062	6,231	6,180
Steer No. 259 (sheared):						
13.68	1a	3,545	747	4,293	4,295	----------
	1b	3,304	663	3,967	4,033	----------
	Total per day	6,849	1,410	8,260	8,328	8,243
15.70	2a	3,113	724	3,837	3,873	----------
	2b	3,178	751	3,929	3,999	----------
	Total per day	6,291	1,475	7,766	7,872	7,889
18.26	3a	2,999	782	3,781	3,813	----------
	3b	2,967	769	3,736	3,727	----------
	Total per day	5,966	1,551	7,517	7,540	7,519
22.00	4a	2,489	904	3,393	3,430	----------
	4b	2,460	862	3,322	3,392	----------
	Total per day	4,949	1,766	6,715	6,822	6,805

DISCUSSION OF RESULTS

The heat production of the fasting animals is recorded in Table 3 by 12-hour subperiods, to permit of judgment of the validity of the heat production of each day as a whole. This is rendered desirable by the fact that the one-day measurements are only one-third the length of the usual calorimeter periods. It was considered impracticable, as well as undesirable, to hold the fasting animal for the three-day period of observation at each of the temperatures.

The agreement between the 12-hour subperiods was usually excellent. With both steers the agreement of these subperiods was least perfect on the first day of the series of measurements, but even on these days it was fair. As a whole, the data are highly satisfactory in spite of the short periods of measurement, one period serving as a check on others in the series.

The heat emission was corrected to the standard day of 12 hours' standing; and then by a correction for the temperature gain or loss in water and excreta, the values for heat emission were computed to heat production. The derivation of the water balance used in this computation is indicated in Table 4.

TABLE 4.—*Experiment 235: Computation of the daily water balance in the fasting experiments with steers*

Animal and period No.	Fresh urine	Dry matter in urine		Water in urine	Fresh feces	Dry matter in feces		Water in feces	Water vapor	Total water outgo	Water drunk	Water balance
Steer 260 (full coat):	*Grams*	*Per cent*	*Grams*	*Grams*	*Grams*	*Per cent*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*
1	1,210	9.15	110.7	1,099	30	[a]34.21	10.3	20	2,690	3,809	6,960	+3,152
2	[b]444	8.02	35.6	408	459	24.52	112.5	347	3,725	4,480	220	−1,260
3	[b]688	------	[c]41.7	647	105	31.98	33.6	71	4,775	5,493	3,590	−1,903
4	1,421	6.06	8.61	1,335	181	37.11	67.2	114	2,616	4,065	2,030	−2,035
Steer 259 (sheared):												
1	2,081	5.91	120.0	1,911	5	[a]27.07	1.4	4	2,391	4,306	[e]920	−3,386
2	6	[d]5.91	.4	6	7	[a]27.07	1.9	5	2,509	2,519	3,300	+781
3	2,111	[e]7.75	163.5	1,948	224	29.61	66.3	158	2,642	4,747	3,760	−987
4	1,785	7.75	138.3	1,647	15	[a]30.80	4.6	10	3,024	4,681	3,770	−911

[a] Assumed to be the same as in samples between periods.

[b] Computed $\frac{41.7\times100}{6.058\text{ (assumed)}}=688.3$.

[c] 1,532 gm. (including wash water) $\times\frac{2.274\text{ (per cent dry matter)}}{100}=41.7$.

[d] Assumed to be the same as in period 1.

[e] Assumed to be the same as in period 4.

In view of the fact that the intake and outgo of water by way of the alimentary and excretory tracts are extremely variable, little evidence of order in the water balances is expected. The outgo of water vapor, however, varies from period to period, always in the same direction as the temperature of the air in the calorimeter chamber. It will be observed that the water balances were negative in six cases of the eight recorded. This is in harmony with past observations at the institute, and perhaps signifies that the drinking facilities in the respiration chamber were not quite satisfactory to the steer. Since a steer can drink, and often does drink, at one draft, a sufficient quantity of water to meet his requirements for two or three days,[3]

[3] The extensive water-drinking capacity of cattle is of great economic importance. In semiarid regions cattle often live month after month without drinking more than two or three times a week. This ability of cattle to go for long periods without drink makes possible the utilization for meat production of forage in regions so remote from markets and so far from water as to be useless for any other agricultural purpose.

it is readily conceivable that a very trifling factor may appreciably affect the water intake and balance.

The heat production in relation to the body surface will be discussed in connection with Table 5. Referring first to the sheared steer, No. 259, it will be noted that in the course of 4 days of observation, beginning with day of fast 5 to 6 and continuing to day 9½ to 10½, with half-day intervals between the 24-hour periods of observation, the temperature of the chamber of the calorimeter was raised by increments of 2.02°, 2.56°, and 3.80°, from 13.68° on day of fast 5 to 6 up to 22.06° on day 9½ to 10½. Thus the changes in temperature were all in one direction—that is, upward.

TABLE 5.—*Experiment 235: Computation of heat production per square meter of body surface of fasting steers*

Animal and period No.	Temperature of chamber	Days of fast	Live weight, fasting	Average live weight, maintenance periods	Empty weight, maintenance periods	Empty weight, fasting	Surface area, $0.1186W^{\frac{2}{3}}$	Heat production per standard day		
								Total	Per 100 kgm. of live weight	Per square meter of body surface
	° *C.*		*Kgm.*	*Kgm.*	*Kgm.*	*Kgm.*	*Sq. m.*	*Cal.*	*Cal.*	*Cal.*
Steer 259 (sheared):										
1	13.68	5 - 6	339.6	350.7	315.6	304.6	4.23	8,243	2,427	1,949
2	15.70	6½- 7½	336.6	350.7	315.6	301.6	4.20	7,889	2,344	1,878
3	18.26	8 - 9	333.6	350.7	315.6	298.6	4.18	7,519	2,254	1,799
4	22.06	9½-10½	330.5	350.7	315.6	295.6	4.15	6,806	2,059	1,640
Steer 260 (full coat):										
1	15.54	4 - 5	356.0	369.3	332.4	323.4	4.39	6,322	1,776	1,440
2	18.46	5½- 6½	353.6	369.3	332.4	320.4	4.37	6,430	1,819	1,471
3	21.51	7 - 8	351.3	369.3	332.4	317.4	4.34	6,453	1,837	1,487
4	14.16	8½- 9½	349.0	369.3	332.4	314.4	4.32	6,180	1,771	1,431
Average, 260 only									1,801	1,457

[a] Moulton's formula (7).

Coincident with each increase in the temperature of the chamber, the animal showed a decrease in heat production. At a temperature of 13.68° C. the heat production was 8,243 Calories; at 15.70°, 7,889 Calories; at 18.26°, 7,519 Calories; and at 22.06°, 6,806 Calories.

Since each increase in temperature was accompanied by a large decrease in heat production, the temperature must have been below the critical for the animal in the first three periods at least; in the fourth period we have no basis for judgment as to the relation of the temperature to the critical.

With steer No. 260, which possessed his full winter coat of hair, the temperature on the four days of observation between days of fast 4 to 5 and 8½ to 9½ was raised from 15.54° to 18.46°, next to 21.51°, and then lowered to 14.16°.

During the periods of increasing temperature the heat production was essentially unchanged (6,322, 6,430, and 6,453 Calories). The slight increase noted from period to period may possibly signify an expenditure of energy in keeping cool, although the evidence on this point is inconclusive. Since these increases in temperature were not accompanied by decreases in heat production, it is obvious that the prevailing temperatures were above the critical for this steer.

After it was observed that the temperature during the first three periods seemed to be above the critical for this steer, it was lowered during the fourth period to 14.16° C. in an effort to reach a temperature below the critical. The heat production at this temperature, however, was the lowest of all. It therefore became perfectly clear that the temperature had been above the critical for this steer in at least the first three periods, and that in the fourth there was as with the other steer, no basis for judgment as to the relation of the temperature to the critical.

Thus the difference in the coat of hair of the two steers led to a sufficient difference in heat production to reveal the environment for one to be clearly above and for the other as clearly below the critical temperature—at the range of temperatures obtainable in the respiration calorimeter as now equipped. It becomes obvious, therefore, that if it is desired to relate the heat production to the feed consumption in work involving low planes of nutrition with cattle the experimental subjects must not be sheared. Fortunately, as already explained, a neglect to account for the growth of the coat does not involve us in significant error, in studies of the body metabolism as a whole.

Another observation of importance which may be made from the heat production of steer No. 260 is that the animal had practically reached the true fasting katabolism on the fourth to fifth day after the last feed.

The influence of the environmental temperature on the manner of the heat loss is shown in Table 6. With both steers the heat lost by radiation and conduction (combined) and that lost by evaporation of water varied in a complementary manner—that is, the warmer the temperature the greater the loss of heat by evaporation and the smaller the loss by radiation and conduction. This relationship prevailed invariably, both as revealed in the absolute quantities of heat and as percentages of the total heat production.

TABLE 6.—*Experiment 235: Influence of temperature on the manner of heat loss*

Animal and period No.	Temperature of chamber	Heat lost by radiation and conduction [a]	Heat lost by evaporation of water	Total heat production [a] [b]	Heat lost by radiation and conduction as per cent of total	Latent heat of water vapor as per cent of total
Steer No. 259 (sheared):	°C.	Cal.	Cal.	Cal.	Per cent	Per cent
1	13.68	6,763.5	1,410.7	8,174.2	82.74	17.26
2	15.70	6,308.7	1,475.0	7,783.7	81.05	18.95
3	18.26	5,944.7	1,550.9	7,495.6	79.31	20.69
4	22.06	4,933.2	1,766.0	6,699.2	73.64	26.36
Steer No. 260 (full coat):						
1	15.54	4,604.2	1,581.4	6,185.6	74.43	25.57
2	18.46	4,081.0	2,186.5	6,267.5	65.11	34.89
3	21.51	3,531.0	2,791.1	6,322.1	55.85	44.15
4	14.16	4,468.2	1,543.5	6,011.7	74.33	25.67

[a] Corrected for gain by body. [b] Not corrected to standard day.

One pronounced difference is noticeable in the response of the two steers to the temperature changes. With about the same range of temperatures, steer No. 260 (full coat) gave off 25.57 to 44.15 per cent of the heat production as latent heat of water vapor, while steer No. 259 (shorn) eliminated 17.26 to 26.36 per cent of the heat in

this manner. This difference is due to the fact that the temperatures were below the critical for steer No. 259. Since this animal had been deprived of its coat of hair, the skin was cooler, the superficial circulation of the blood was doubtless diminished by the contraction of the capillaries, and the outgo of latent heat of water vapor was naturally lessened.

Thus far we have dealt with the heat production of the individual animal, computed to a standard day as to standing and lying, but no effort has been made to express the heat in relation to a unit of size of the animal. Two practicable units of reference are those representing live weight and surface area. Each has its point of superiority. Thus, live weight can be ascertained with relative accuracy, and without extensive compromise as to significance on account of variation in the content of the alimentary and excretory system, but it has the disadvantage of not varying directly as the heat production. Surface area, on the other hand, has the advantage of varying more nearly as does the heat production; but in using this measure one is handicapped by the fact that there is no perfect means for determining the surface area of the animal.

Among the possible means for determinging the surface area are (1) computation in accord with the two-thirds power of the live weight; (2) direct measurement on the living animal, as by Brody's "surface integrator"; and (3) reference to formulae, based either upon Brody's surface measurements or upon measurements of the freshly removed skins of animals of the kind used in the experiment. There are obvious possibilities of error in all of these methods. In this study the heat production is expressed (1) per head, (2) per kilogram of live weight, and (3) per square meter of body surface, the surface area being computed as in Table 5.

In this computation, beginning with the average live weights as observed in maintenance periods, the empty live weights in maintenance periods were computed by the use of the factor 0.9, taken from the work of Trowbridge, Moulton, and Haigh (*8*). Next, from these empty live weights during maintenance were computed the empty live weights during fast, by subtracting from the former values the estimated loss of body tissue during fast. In this computation the protein loss was computed from the urinary nitrogen, and the carbon balance was computed to fat, the carbon lost as carbon dioxide plus the carbon in the urine being considered as the total loss of carbon derived from body tissue.

From the empty live weights during fast the surface areas during fast were computed by the use of Moulton's formula $0.1186W^{\frac{2}{3}}$ (7). The most obvious possibility of error in this procedure seems to be in the measurements of the hides, as indicating the surface area of the animals, the uncontrolled factors being the folding of the skin and its tension. Not only does the skin lie to some extent in folds on the live animal, but obviously a difference in tension must accommodate the difference in surface area caused by the "fill" of the animal.

The heat production on the three above-mentioned bases is presented in the three columns at the right in Table 5. Average values for steer No. 259 are not presented, since these would be without definite significance in relation to a normally covered animal, but for steer No. 260 averages of 18.01 Calories per kilogram of live weight and 1,457 Calories per square meter of body surface are presented as

applying to normal cattle. The latter datum is of especial significance and value in the computation of the maintenance requirement of energy.

The decrease in heat production per square meter of body surface per degree of rise in temperature, as observed with steer No. 259, and derived as indicated in Table 7, is a figure of fundamental significance, especially as indicating the rate of loss of heat, or saving of heat (according to circumstances), as affected by temperatures below the critical. On the basis of the evidence derived from this experiment it appears that a rise of 1 degree in temperature below the critical occasions a saving of 36 Calories per square meter of body surface.

TABLE 7.—*Experiment 235: Effect of subcritical temperatures on the daily heat production of steer No. 259*

Periods compared	Rise in temperature of chamber	Decrease in heat production per square meter of body surface	Decrease in heat production per square meter of body surface per degree of rise in temperature
	° *C.*	*Calories*	*Calories*
1 and 2	2.02	71	35
1 and 3	4.58	150	33
1 and 4	8.38	309	37
2 and 3	2.56	79	31
2 and 4	6.36	238	37
3 and 4	3.80	159	42
Average			36

The practical significance of this fact, which is so clear as to require no special demonstration, is that under the usual conditions of intensive cattle feeding, where feed and shelter are to be had at reasonable cost, it is more profitable to keep the animal above the critical temperature than to force it to burn extra feed or to draw upon body reserves of energy in order to keep warm. Whatever the conditions as to profitable practice, however, it is important to know that a fall of 1° C. in temperature below the critical increases the cost of maintenance of cattle by about 2.5 per cent.

Use may also be made of the value for decrease in heat production per square meter of body surface per degree of rise in temperature below the critical in computing the critical temperature of an animal. In order to do this it is necessary to have the heat production at two points, one above and one distinctly below the critical temperature. By such procedure it is possible to study the effect of various factors of treatment on the critical temperature. The data in Table 7 were obtained with an approximately half-fat steer weighing 335 kgm. No information is available to show the accuracy with which the result determined would apply to animals of other weights and degrees of fatness.

CONCLUSIONS

A recent review and recalculation of published energy metabolism work of this institute has brought to light the fact that in one experiment at least, and perhaps in others, there was a confusion of heat produced from the feed with heat produced from the katabolism of body tissue, by reason of the fact that the shearing of the steer which served as the experimental subject had the effect of raising the critical temperature for the animal above the temperature of the chamber of the respiration calorimeter.

A steer receiving as much as 2.933 kgm. of dry matter of feed per day may find a temperature of 19° C. below its critical temperature if the animal has been sheared.

In a fasting experiment, with a steer, beginning four days after the last feed was given, the heat production on the first day of observation—that is, on day 4 to 5 after feeding—was found to represent essentially the true fast.

In the case of a fasting steer, shorn of its coat of hair, weighing 339.6 to 330.5 kgm., at environmental temperatures of 13.68°, 15.70°, 18.26°, and 22.06° C., the 24-hour heat production was, respectively, 8,243 Calories, 7,989 Calories, 7,519 Calories, and 6,806 Calories, showing that at least the first three temperatures were below the critical for this animal.

With a fasting steer carrying a full coat of hair, weighing 356 to 349 kgm., and exposed to environmental temperatures of 15.54°, 18.46°, 21.51°, and 14.16° C., the corresponding 24-hour heat production was 6,322 Calories, 6,430 Calories, 6,453 Calories, and 6,180 Calories, respectively, at least the first three temperatures being above the critical. The data suggest that at these temperatures there may have been a slight expenditure of energy by the animal in keeping cool.

With both the shorn steer and the steer with a full coat the percentage of the heat emission as latent heat of water vapor increased with the increase in temperature, this effect being much more pronounced with the steer having the full coat of hair. Conversely, the percentage of the heat emission lost by radiation and conduction (combined) decreased with increase in the temperature.

The average heat production of a fasting steer with its full winter coat was 18.01 Calories per kgm. of live weight and 1,457 Calories per square meter of body surface.

The decrease of heat production per degree centigrade of rise in temperature below the critical was 36 Calories per square meter of body surface, or about 2.5 per cent of the fasting katabolism.

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THE FASTING KATABOLISM OF DRY COWS[1]

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INTRODUCTION

This paper presents results on one aspect of a general investigation of the energy metabolism of milk production. The research as a whole was planned by the late H. P. Armsby, former director of the Institute of Animal Nutrition, Pennsylvania State College, the experiments here discussed having been conducted in the years 1919 to 1922, under the immediate direction of J. August Fries, with the cooperation of W. W. Braman, D. C. Cochrane, Max Kriss, C. D. Jeffries, W. J. Sweeney, and R. M. Meredith. The reader is referred to other papers (*4, 1, 2, 3*)[2] which have been published on this project.

The fasting katabolism of dry cows is of special interest in connection with the maintenance requirement in lactation, and this paper may be regarded as introductory to a paper (*3*) which is to follow, which is based upon studies with the same individual animals in experiments on the production of milk.

In utilizing the directly determined fasting katabolism as a measure of the maintenance requirement of energy the writers are departing from the previous method of the institute, which, following Armsby, was to compute the fasting katabolism (maintenance requirement) from the difference in heat production resulting from a corresponding difference in feed at two planes of feeding. The evidence which seems to the writers to justify this change, and a discussion of the whole subject of determination of the maintenance requirement of energy, will be presented at an early date. Suffice it to say, for the present, that the new procedure referred to simply involves the use of a directly determined instead of a computed measure, without change in theory, and that the new procedure brings this institute into harmony, in this regard, with the students of the energy metabolism of animals generally. However, the use of the writers' new measure of the maintenance requirement for cattle involves most important consequences in the determination of net-energy values.

It will readily be appreciated that in making use of the fasting katabolism as a routine measure it is necessary that it be determined in a standard way. The evidence necessary for the establishment of such a standard procedure is not yet at hand, but will be at an early date; and the present paper is a contribution toward this object.

A question which naturally suggests itself to anyone who is interested in the fasting katabolism in connection with the practical problems of animal feeding is as to the real significance of this measure

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[2] Reference is made by number (italic) to "Literature cited," p. 595.

in such relations. Is the heat production of a starving animal wholly the result of normal processes, or is the starving animal's metabolism essentially abnormal and deranged, in such sense that it can not be regarded as the true maintenance quota of net energy during feeding?

The metabolism of fasting is characterized by several conditions, which, whether they be regarded as normal or abnormal, do differ distinctly from those prevailing during feeding. Thus, during fast there is, of course, a depletion of not only the body content of carbohydrate and fat, but of all essential nutrients, including proteins, mineral salts, and vitamins; also there is commonly a negative water balance, at least a mild degree of acidosis, and Lennox (*6*) now reports a retention of uric acid.

Furthermore, rations for cattle always contain a much larger proportion of carbohydrate in comparison with fat and protein than that present in the body nutrients katabolized by fasting cattle; and, since each of these classes of nutrients is utilized at its own individual rate of efficiency, it follows that the heat production of fasting must stand in a quantitative relation to the nutrients consumed different from that prevailing during the use of any normal ration.

As for the nature of the influence, upon the associated heat production, of the several conditions of metabolism which are characteristic of fasting, the existing knowledge is far from complete but is sufficient perhaps to justify the discussion of the conception of a hypothetical "normal" maintenance requirement of energy differing in amount from the observed fasting katabolism which the writers are adopting as their measure of the maintenance quota.

The writers have found, in experiments the results of which are as yet mostly unpublished, that when they compute the fasting katabolism (as a measure of the maintenance requirement) from the difference in heat production at two supermaintenance levels of feed intake, they obtain a value which is significantly lower than the directly determined fasting katabolism. This shows that supermaintenance heat increments are larger than submaintenance increments, or, in other words, that the heat increment is not a straight-line function of the amount of the feed, and, consequently, that it is improper thus to use supermaintenance increments to apply to submaintenance conditions. Whether the hypothetical "normal" maintenance requirement of energy would be the same as or higher or lower than the fasting katabolism (maintenance requirement) as computed from supermaintenance heat increments is, in the light of the present limited knowledge, a debatable question; that is, it is not known whether supermaintenance heat increments are higher than submaintenance increments on account of heat-stimulating effects of "abnormal" or other characteristic conditions of fasting, or on account of actual differences in utilization of feed energy, because the shape of the curve of heat increments in relation to feed consumed, from a state of fast up to maximum feed consumption, is not known.

However, from the point of view of the writers' present interest in the significance of the fasting katabolism as related to metabolism during feeding, they dismiss the whole subject of the origin, composition, and essential nature of the fasting katabolism (as to its being "normal" or "abnormal"), because, whatever the facts in this situation, the amount of food which is capable of entirely preventing this katabolism of body substance (during fast) must be

credited with a net-energy value measured by the protection thus afforded the body from loss; in other words, the heat production of fasting, which represents the loss of energy by the body, is the measure of the net-energy value of a ration required to keep the animal in energy equilibrium.

In this light the fasting katabolism may be regarded as of direct and unqualified significance as a measure of the maintenance requirement of energy, in relation to feeding practice.

EXPERIMENTAL DATA

Referring to Table 1, and proceeding from the left to the right in discussing the data, the first column signifies that in experiments 221D, 221E, and 221F, cows 886, 885, 874, and 887 were subjected to respiration calorimetric studies, two on the second and third days of fast, one on the fifth and sixth days of fast, and two on days 6½ to 7½ and 7½ to 8½.

TABLE 1.—*Energy metabolism of fasting dry cows, computed to calories per square meter of body surface*

Experiment; animal; period; and day of fast	Live weight, fasting	Live weight, maintenance periods	Empty live weight, maintenance periods	Empty live weight, fasting (W)	Body surface (0.1186 ×W⅔)	Heat production per day
1	2	3	4	5	6	7
221D, 886, IV:	*Kgm.*	*Kgm.*	*Kgm.*	*Kgm.*	*Sq. m.*	*Cals.*
Day 2-3	411	420.2	378.2	376.2	4.83	6,752
Day 3-4	405			374.2	4.81	6,336
221D, 885, IV:						
Day 2-3	420	426.4	383.8	381.8	4.87	6,760
Day 3-4	417			379.8	4.86	6,570
221E, 885, III:						
Day 5-6	421	434.4	391.0	383.0	4.88	6,601
Day 6-7	419			381.0	4.87	6,576
221F, 874, III:						
Day 6½-7½	395	415.6	374.0	361.0	4.70	6,996
Day 7½-8½	393			359.0	4.69	6,658
221F, 887, III:						
Day 6½-7½	301	320.5	288.5	275.5	3.97	6,019
Day 7½-8½	297			273.5	3.96	6,228

Experiment; animal; period; and day of fast	Heat production, standard day	Heat production, per square meter of body surface	Heat production, per kilogram of body weight	Latent heat of water vapor	Temperature of chamber
	8	9	10	11	12
221D, 886, IV:	*Cals.*	*Cals.*	*Cals.*	*Per cent*	*°C.*
Day 2-3	6,779	1,404	16.49	20.72	17.54
Day 3-4	6,400	1,331	15.80	20.72	17.54
221D, 885, IV:					
Day 2-3	6,523	1,339	15.53	26.38	17.63
Day 3-4	6,374	1,312	15.29	25.31	17.59
221E, 885, III:					
Day 5-6	6,384	1,308	15.16	22.39	18.41
Day 6-7	6,413	1,317	15.31	22.14	18.45
221F, 874, III:					
Day 6½-7½	6,906	1,469	17.48	21.47	17.94
Day 7½-8½	6,669	1,422	16.97	22.48	17.98
221F, 887, III:					
Day 6½-7½	5,778	1,455	19.20	19.72	17.92
Day 7½-8½	5,982	1,511	20.14	18.77	17.90

In all cases the animals were on practically a maintenance basis of nutrition when the fasting experiments began. In all cases the rations were composed of 40 per cent of alfalfa hay and 60 per cent of a grain mixture consisting of 30 per cent corn meal, 30 per cent wheat bran, 30 per cent ground oats, and 10 per cent linseed meal. The kilograms of dry matter of this ration fed to each of the cows during the preliminary treatment are the following: Cow 886, 3.782; cow 885 (experiment 221D), 3.851; cow 885 (experiment 221E), 3.448; cow 874, 4.003; and cow 887, 3.627.

In view of the differences in the length of these fasting experiments, from 3 to 8½ days, it is obvious that the results are not perfectly comparable. However, the agreement of the results, shows that in spite of the differences in length of fasts, the heat production was in no case very different from that in any other.

The live weights as observed in the fasting periods comprise the second column of Table 1, but inasmuch as the heat production varies more nearly with the surface area than with the live weight it is necessary to compute the body surface for each cow in each period. This computation is outlined in the next four columns of figures in Table 1. The third column gives the observed live weights in the maintenance periods. The fourth column gives the empty live weights in the maintenance periods, these weights having been computed from the live weights, as observed, by the use of the factor 0.9 taken from the work of Trowbridge, Moulton, and Haigh (*8*). The empty live weights, fasting, (fifth column) were computed by subtracting from the weights in the fourth column the computed loss of tissue during fast. The body surface (sixth column) was computed from the figures in the fourth column by the use of Moulton's formula, $0.1186 \times W^{5/8}$ (*7*). The writers are fully conscious of the considerable measure of assumption involved in this computation of the surface area of their experimental subjects, but are unable to do better at this time.

The seventh, eighth, ninth, and tenth columns set forth the heat production, on various bases. The seventh column is the heat production as observed, by direct calorimetry. In the eighth column this heat production is computed to the standard day, as to standing and lying, by the method of Fries and Kriss (*5*). The ninth column is the heat production per square meter of body surface In the tenth column the heat production is related to the live weight.

In the eleventh and twelfth columns are set forth the latent heat of water vapor as per cent of the total heat production, and the temperature of the chamber of the calorimeter, these data being recorded on account of the possibility of their being of interest in relation to the critical temperature.

The most significant data presented here are those in the ninth column, representing the heat production in Calories per square meter of body surface. In studying these figures it is necessary to keep in mind the fact that in four of the five fasting periods the subjects were different individuals, and that the two fasting experiments with cow 885 were separated by an interval of about a year; also it should be understood that the data for heat production cover not only the basal metabolism, but, in addition, the normal activity of the cows, as when confined to a stall.

With both cows 886 and 885 the heat production on the third day of fast was less than on the second; and with cow 885 the heat production on the third day was essentially the same as on the fifth and sixth days.

With cows 874 and 887 the heat production on days 6½ to 7½ and 7½ to 8½ of fast was higher than that of cows 886 and 885 at any time. Presumably this higher production was due not to the greater duration of the fast but to individuality.

While these results are obviously promising, the writers refrain from drawing conclusions until they can be considered together with later data of similar character which will soon be available.

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